Modelling the effect of specific inositol 1,4,5-trisphosphate receptor isoforms on cellular Ca^{2+} signals

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Background information. Oscillations of cytosolic Ca^{2+} are well-known to rely on the regulatory properties of the $InsP_3R$ (inositol 1,4,5-trisphosphate receptor). Three isoforms of this channel have been identified. They differ in their regulatory properties by Ca^{2+} and $InsP_3$. Experiments in different cell types clearly indicate that the relative amounts of each isoform affect the time course of Ca^{2+} changes after agonist stimulation. In the present study, we investigate whether different steady-state curves for the open probability of the $InsP_3Rs$ as a function of Ca^{2+} imply different dynamical behaviours when these receptors are present in a cellular environment. We therefore describe by a specific phenomenological model the three main types of curves that have been reported: (i) the classical bell-shaped curve, (ii) the bell-shaped curve that is shifted towards higher Ca^{2+} concentrations when $InsP_3$ is increased, and (iii) a monotonous increasing function of cytosolic Ca^{2+}.

Results. We show that, although these types of curves can be ascribed to slight differences in the channel regulation by Ca^{2+} and $InsP_3$, they can indicate important variations as to the receptor role in cellular Ca^{2+} control. Thus the receptor associated with the classical bell-shaped curve appears to be the most robust Ca^{2+} oscillator. If the steady-state curve is supposed to be a monotonous increasing function of cytosolic Ca^{2+}, the modelled receptor cannot sustain Ca^{2+} oscillations in the absence of Ca^{2+} exchanges with the extracellular medium. When the bell-shaped curve is shifted towards higher Ca^{2+} concentrations with increasing $InsP_3$ levels, the model predicts that the receptor is less robust to changes in density; this receptor, however, provides a finer control of the steady-state level of Ca^{2+} when varying the $InsP_3$ concentration.

Conclusions. Our model allows us to propose an explanation for the experimental observations about the effect of selectively expressing or down-regulating $InsP_3R$ isoforms, as well as to make theoretical predictions.

Introduction

In many cell types, stimulation by an external agonist induces the synthesis of $InsP_3$ (inositol 1,4,5-trisphosphate) and the subsequent mobilization of Ca^{2+} ions from the intracellular Ca^{2+} stores. As the $InsP_3Rs$ ($InsP_3$ receptors)/Ca^{2+} channels are also regulated by Ca^{2+}, they play a primary role in the generation of the $InsP_3$-induced Ca^{2+} oscillations, which in turn control a vast array of cellular functions

(Berridge et al., 2000; Combettes et al., 2004). It is often assumed that the potency of the cell to control diverse physiological processes with a compound as simple as Ca^{2+} ions results from the large versatility of the signal-induced Ca^{2+} changes (Berridge et al., 2000). The existence of different $InsP_3R$ isoforms is probably an important factor allowing such diversity of responses (Ramos-Franco et al., 1998; Miyakawa et al., 1999; Haberichter et al., 2002; Yule et al., 2003; Hattori et al., 2004; Morita et al., 2004; and reviewed in Vermassen et al., 2004).

In mammalian cells, three $InsP_3R$ subtypes have been identified: $InsP_3R1$, $InsP_3R2$ and $InsP_3R3$. In all cell types, functional channels result from the

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assembly of receptors into homo- or hetero-tetrameric structures. The three isoforms are co-expressed within cells, but their respective levels of expression are largely tissue- and development-specific (De Smedt et al., 1997; reviewed in Taylor et al., 1999, and Vermassen et al., 2004). Although all the InsP$_3$R subtypes display similar ion permeation properties, they differ significantly in their regulatory properties, due to different interactions with accessory proteins, various modes of regulation by ATP or phosphorylation (Kaftan et al., 1997; Hagar et al., 1998; Wojcikiewicz and Luo, 1998; Moraru et al., 1999; Mak et al., 2001a, 2001b; Patterson et al., 2004). The exact nature of these differences, as well as their molecular origin, however, remains controversial (reviewed in Taylor and Laude, 2002, and Patterson et al., 2004).

In a cellular environment, the respective proportions of the three InsP$_3$Rs affect the time course of cytosolic Ca^{2+} concentration after agonist stimulation. Thus in DT40 B-cells expressing a single InsP$_3$R subtype, the Ca^{2+} signals in response to BCR (B-cell receptor) stimulation is highly dependent on the receptor subtype (Miyakawa et al., 1999). Strikingly, only the InsP$_3$R2 isoform appears to be able to generate long-lasting, regular Ca^{2+} oscillations in this cell type. Similar results were obtained in myocytes (Morel et al., 2003). In other studies (Hattori et al., 2004), levels of InsP$_3$R1 or InsP$_3$R3 were knocked down by RNAi (RNA interference) in HeLa and COS-7 cells. In both cell types, knockdown of InsP$_3$R1 tends to decrease the number of Ca^{2+} spikes, whereas knockdown of InsP$_3$R3 has the opposite effect and tends to favour long-lasting Ca^{2+} oscillations. Together, these experiments thus demonstrate a close correlation between the types of InsP$_3$Rs present in a cell and the existence (and characteristics) of InsP$_3$-induced Ca^{2+} oscillations.

From a modelling point of view, it has already been suggested that variable ratios between type 1 and type 3 InsP$_3$Rs may explain why the airway smooth muscle cells do not all display Ca^{2+} oscillations in response to the same stimulus (Haberichter et al., 2002). It is proposed in the study by Haberichter et al. (2002) that slight, random, inter-individual variations in the ratios of InsP$_3$R1 to InsP$_3$R3 suffice to account for the different sensitivities of these cells to agonist stimulation. Other models have considered only one isoform, but have shown that the specificity

of the major InsP$_3$R subtype present in a given cell type can explain cell-specific characteristics of Ca^{2+} oscillations. For example, the PKA (protein kinase A)-induced phosphorylation of the InsP$_3$R3 could be responsible for the typical shape of the CCK (cholecystokinin)-induced Ca^{2+} oscillations in pancreatic acinar cells (LeBeau et al., 1999). Specific modelling of the InsP$_3$R2 has also been considered in the case of rat adrenal chromaffin cells (Inoue et al., 2003).
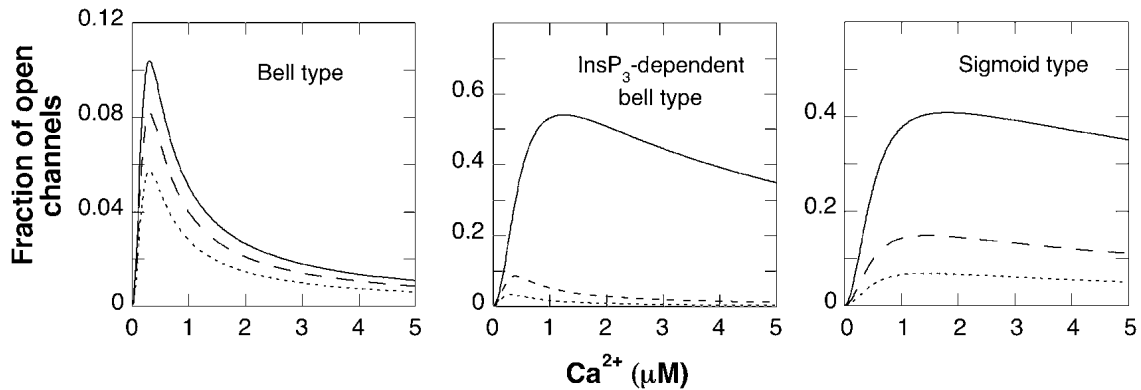
In the present work, we have theoretically studied the possible implications of the different regulations by InsP$_3$ and Ca^{2+} of the various InsP$_3$ isoforms on the global Ca^{2+} dynamics. To this end, we first develop phenomenological models accounting for the distinct steady-state behaviours that have been reported in the literature. Due to the absence of consistent experimental data, we do not try to relate a given type of steady-state curve to a specific InsP$_3$ isoform. We rather focus on the relation between a given type of steady-state behaviour of an InsP$_3$R and its characteristics in the generation or shaping of Ca^{2+} oscillations. Our models are phenomenological in the sense that they are not based on the underlying molecular processes related to InsP$_3$ and Ca^{2+} binding, but are rather mathematical expressions fitting the observed behaviours. We then incorporate these different descriptions of the InsP$_3$Rs into a model for the dynamics of cellular Ca^{2+} signalling. We find that slight differences in the Ca^{2+} regulatory properties of these InsP$_3$Rs/Ca^{2+} channels can lead to drastically distinct Ca^{2+} signalling patterns when they are expressed in a cell. Our results provide a possible interpretation of the experimental results obtained in myocytes (Morel et al., 2003), DT40 B-cells (Miyakawa et al., 1999), HeLa and COS-7 cells (Hattori et al., 2004) as to the effect of selectively expressing or down-regulating InsP$_3$R isoforms.

Mathematical model

The steady-state open probability of the InsP$_3$R/Ca^{2+} channel presents a bell-shaped dependence on the cytosolic Ca^{2+} concentration (Bezprozvanny et al., 1991; Finch et al., 1991). Although steady-state properties are insufficient to fully characterize the properties of a channel (Sneyd and Dufour, 2002), this bell-shaped curve most probably results, in fact, from a fast activation of Ca^{2+} flux by cytosolic Ca^{2+}, followed by a slower inhibition. However, the details

Figure 1 | Steady-state open probabilities of the different types of InsP₃Rs as a function of the concentration of Ca²⁺ at the cytosolic side of the channel, for three concentrations of InsP₃: 0.2 μM (dotted line), 0.5 μM (dashed line) and 2 μM (continuous line)

Bell type: curves were obtained by solving eqns (1) and (2) at steady state, with the following parameter values: $k_+^B = 14.4606\ \mu M^{-3} \cdot s^{-1}$, $k_-^B = 0.217\ s^{-1}$, $K_{act} = 0.5\ \mu M$, $K_B = 0.2\ \mu M$, $n_i = 3$, $n = 3$, $n_a = 2$. The inhibition constant of the receptor by Ca²⁺ was calculated by $K_{inh}^B = (k_-^B / k_+^B)^{1/n_i} = 0.247\ \mu M$. InsP₃-dependent bell type: curves were obtained by solving eqns (3) and (4) at steady state, with the same parameter values as for the bell type, except for: $K_i^I = K^I = 0.5\ \mu M$. Sigmoid type: curves have been obtained by solving eqns (1) and (2) at steady state, with the same parameter values as for the InsP₃-dependent type, except for: $k_-^S = 21.7\ s^{-1}$, $K_i^S = K^S = 4\ \mu M$. Thus $K_{inh}^S = 1.145\ \mu M$.

of this dual regulation differ significantly from one study to the other (Taylor and Laude, 2002). In attempting to classify the behaviours reported in the literature, one can distinguish the three following cases.

(i) A classical fast activation–slow inhibition of the Ca²⁺-releasing activity of the InsP₃R by Ca²⁺, with apparently no interplay between the regulations by Ca²⁺ and by InsP₃ (Champeil et al., 1989; Dufour et al., 1997; Ramos-Franco et al., 1998). Here, we will call this type of receptor the 'bell type', to refer to the classical bell-shaped curve which is characteristic of its open probability. Many mathematical models have been proposed to account for such a behaviour (Schuster et al., 2002). Here, we use a simple, previously published description for this type of receptor dynamics (Dupont and Swillens, 1996). In this first approach, we indeed do not analyse the different assumptions about the dynamics of the channel that could lead to similar steady-state curves. In this model, the evolution equation for R_i^B, defined as the fraction of InsP₃R of the bell type that has been inhibited by Ca²⁺, is given by:

$$\frac{dR_i^B}{dt} = k_+^B \left(1 - R_i^B\right) \cdot \frac{C_c^{n_i}}{1 + \left(\dfrac{C_c}{K_{act}}\right)^{n_a}} - k_-^B R_i^B \quad (1)$$

where k_+^B is the rate of inhibition of this InsP₃R type by cytosolic Ca²⁺ and k_-^B is the rate of relief from this inhibition. Activation of the receptor by Ca²⁺ is assumed to be instantaneous and characterized by a threshold constant K_{act}. Inhibition is assumed to occur only on receptors that have been activated by Ca²⁺. Parameters n_a and n_i are the Hill coefficients, characterizing the co-operativity of the activation and inhibition processes. The fraction of active (i.e. open) receptors of this type is then given by:

$$IR_a^B = \left(1 - R_i^B\right) \cdot \frac{IP_3}{K_B + IP_3} \cdot \frac{C_c^{n_a}}{C_c^{n_a} + K_{act}^{n_a}} \quad (2)$$

where K^B is the half-saturation constant of the bell-type InsP₃R for InsP₃ (see below for the value of this parameter). The InsP₃ dependence of Ca²⁺ release is proposed to be non-cooperative. The fraction of open InsP₃R of this bell type at steady state as a function of Ca²⁺ concentration is shown in Figure 1 (left-hand panel) for three values of the InsP₃ concentration. As expected from eqn (2), an increase in InsP₃ simply shifts the bell-shaped curve towards higher opening levels (but not horizontally).

(ii) In other experiments, an interplay between the regulations by Ca²⁺ and InsP₃ have been reported

in addition to the biphasic regulation of the receptor activity by Ca^{2+}. In some instances, indeed, the threshold Ca^{2+} concentration leading to $InsP_3R/Ca^{2+}$ channel inhibition increases with the $InsP_3$ concentration (Kaftan et al., 1997; Mak et al., 1998; Moraru et al., 1999). We refer to this behaviour as to the '$InsP_3$-dependent bell type'. Sophisticated models based, for example, on the existence of two $InsP_3$-binding sites characterized by different affinities have been proposed (Kaftan et al., 1997). In the present study, we simply assume that the inhibition of this $InsP_3R$ by Ca^{2+} is reduced when the level of $InsP_3$ increases. This approach is, in fact, similar to that of Mak et al. (1998), where the half-saturation constant for inhibition of the $InsP_3R$ by Ca^{2+} increases with $InsP_3$ [eqn (2) in Mak et al., 1998]. A plausible molecular explanation would be that the Ca^{2+} inhibition of the $InsP_3R$ is mediated by calmodulin. Following this hypothesis, the ability of $InsP_3$ to protect receptors from Ca^{2+} inhibition would reflect its ability to regulate the interplay between calmodulin and the inhibitory Ca^{2+}-binding sites (reviewed in Taylor and Laude, 2002). The evolution equation for R_i^I, defined as the fraction of $InsP_3R$ of the $InsP_3$-dependent bell type that has been inhibited by Ca^{2+}, is given by:

$$\frac{dR_i^I}{dt} = k_+^I \left(1 - R_i^I\right) \cdot \frac{C_c^{n_i}}{1 + \left(\frac{C_c}{K_{act}}\right)^{n_a}}$$

$$\cdot \frac{\left(K_i^I\right)^n}{\left(K_i^I\right)^n + (InsP_3)^n} - k_-^I R_i^I \qquad (3)$$

where k_+^I is the rate of inhibition of this type of $InsP_3R$ by cytosolic Ca^{2+} and k_-^I the rate of relief from this inhibition. K_i^I and n are the threshold constant and Hill coefficient characterizing the inhibitory effect of $InsP_3$ on the Ca^{2+}-induced inhibition of the receptor. As in the case of the other receptor type, the fraction of active receptors is given by:

$$IR_a^i = \left(1 - R_i^I\right) \cdot \frac{InsP_3}{K^I + InsP_3} \cdot \frac{C_a^{n_a}}{C_a^{n_a} + K_{act}^{n_a}} \qquad (4)$$

As shown in Figure 1 (middle panel), the fraction of open $InsP_3R$ calculated on the basis of eqns (3) and (4) first increases and then decreases when increasing the level of Ca^{2+} at the cytosolic side of the channel, with a shift of the maximum of the curve to the right when

increasing $InsP_3$ concentration. The maximum open probability also much depends on the $InsP_3$ concentration; this is due to the decreased level of inhibition by Ca^{2+} at high $InsP_3$ concentration. When comparing the classical type of receptor with that taking into account the interaction between $InsP_3$ and Ca^{2+}, it is noticeable that the second type is less sensitive to Ca^{2+} changes. Receptor inhibition by Ca^{2+} is indeed less effective when this inhibition is prevented by $InsP_3$.

(iii) A third type of behaviour that has been reported in the literature is that of an $InsP_3R$ which is activated by Ca^{2+}, but inhibited by this compound only at very high Ca^{2+} concentrations (Hagar et al., 1998; Miyakawa et al., 1999). In this case, there is also an interplay between $InsP_3$ and Ca^{2+}, clearly visible from the shift of the open probability curve to the right when increasing $InsP_3$ (Mak et al., 2001b). As the open probability of the $InsP_3R$ approximately increases with Ca^{2+} in a sigmoidal manner (at least for Ca^{2+} concentrations at 0.1–5 µM), we refer to this as the 'sigmoid type'. We consider for this type the same evolution equation as for $InsP_3$-dependent bell type [eqn (3) where the index I is replaced by S], but change the value of the kinetic constant k_- so that k_-^S is 10 times larger than k_-^I. In such conditions, inhibition by Ca^{2+} is much less effective. Thus when plotting the fraction of open $InsP_3R$ of the sigmoid type as a function of the cytosolic Ca^{2+} concentration, the biphasic character or the curve nearly disappears (Figure 1, right-hand panel). Note that the difference between the curves aimed at modelling the $InsP_3$-dependent bell and the sigmoid types is mainly visible at low $InsP_3$ concentrations. Indeed, when $InsP_3$ is high, the inactivation by Ca^{2+} plays a minor role in both cases.

The various types of $InsP_3Rs$ are not only differently regulated by Ca^{2+}, they also bind $InsP_3$ with different affinities. These differences influence the potency of $InsP_3$ to release Ca^{2+} (Missiaen et al., 1998; Wojcikiewicz and Luo, 1998; Miyakawa et al., 1999; Dyer and Michelangeli, 2001). In our model, these differences are reflected by distinct half-saturation constants for $InsP_3R$ activation by $InsP_3$. In agreement with experimental data, we selected $K^B = 0.2$ µM, $K^I = 0.5$ µM and $K^S = 4$ µM for the bell, $InsP_3$-dependent bell and sigmoid types respectively. It should be emphasized that our description of the regulation of the various $InsP_3R$ subtypes is

much simplified and mainly dictated by the $InsP_3$- and Ca^{2+}-dependence of the differentially expressed $InsP_3R$ isoforms in DT40 B-cells (Miyakawa et al., 1999), as they are directly related to the Ca^{2+} signalling data.

To test the effect of realistic variations in the mode of regulation of the $InsP_3Rs$ on the global Ca^{2+} dynamics in a cell, we have to incorporate our equations describing the dynamics of the three receptor subtypes [eqns (1)–(4)] into a global description of the Ca^{2+} exchange processes between the cytosol and the ER (endoplasmic reticulum). Thus Ca^{2+} is released from the ER by three types of $InsP_3R$ that can possess different maximal fluxes and/or are present in various amounts. Here, we consider both effects together as we are interested in the participation of one given type of receptor on the global Ca^{2+} dynamics. Thus we introduce the parameters λ^B, λ^I and λ^S to quantify the maximal Ca^{2+} fluxes through the bell, $InsP_3$-dependent bell and sigmoid $InsP_3R$ types respectively. Pumping of Ca^{2+} back into the ER is represented by the usual kinetic expression for a Ca^{2+}-ATPase. Thus the evolution of the concentration of cytosolic Ca^{2+} in a cell is:

$$\frac{dC_c}{dt} = \sum_{i=1}^{3} \lambda^i (b + IR^i) \cdot [C_T - C_c(\alpha + 1)]$$

$$- V_{MP} \frac{C_c^{n_P}}{C_c^{n_P} + K_P^{n_P}} \qquad (5)$$

where the i stands for B, I or S. C_T stands for the total concentration of free Ca^{2+} in the cell, α for the volume ratio between the ER and the cytosol; $\lambda^i b$ represents a small leak term through the different receptor types.
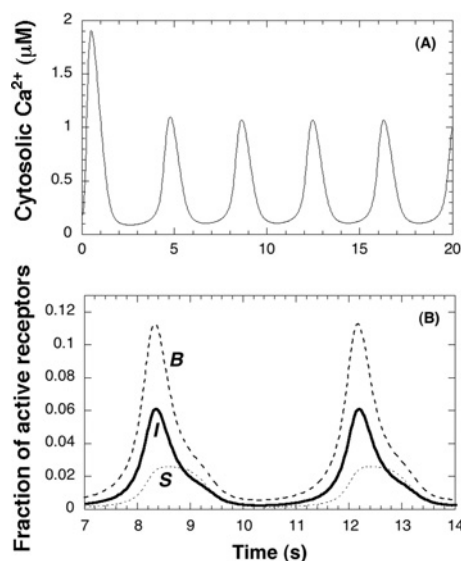
Results

Different dynamics of the three receptor types

Figure 2(A) shows simulated Ca^{2+} oscillations in a cell expressing the three types of $InsP_3Rs$. For the chosen amounts of receptor types, sustained oscillations are observed for $InsP_3$ concentrations from 60 to 260 nM. It is clear from Figure 2(B) that the three isoforms exhibit different behaviours in the course of Ca^{2+} oscillations. The highest fraction of open receptor is reached by the type exhibiting a classical bell-shaped curve at steady state [dashed line in Fig-

Figure 2 | Oscillations in the concentration of cytosolic Ca^{2+} (A) and in the fraction of open $InsP_3Rs$ (B) in a simulated cell assumed to co-express all three $InsP_3R$ isoforms
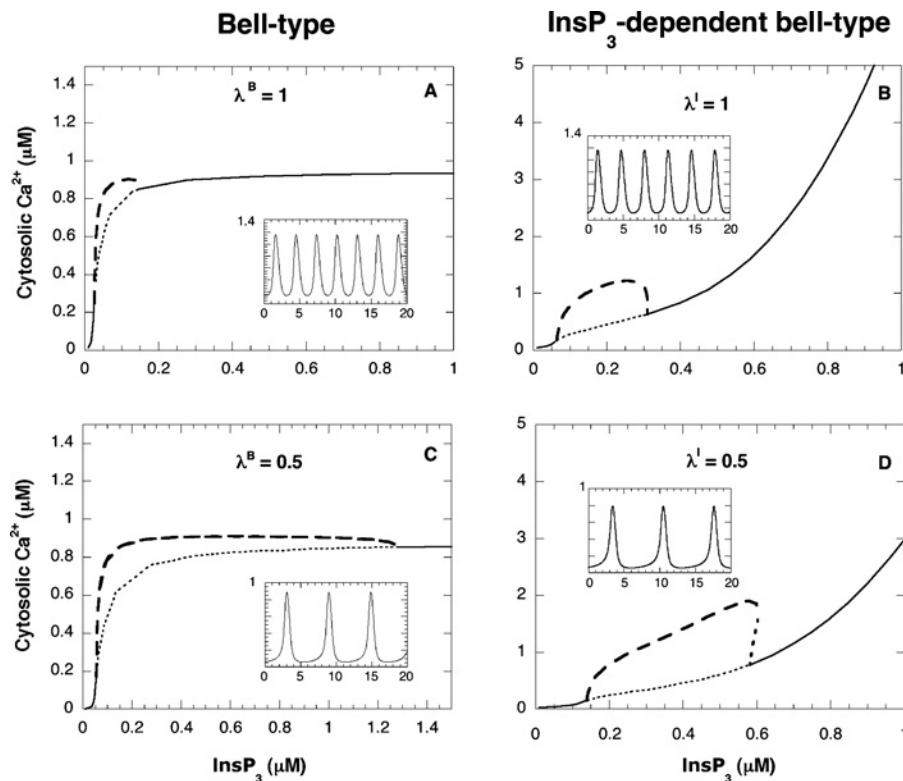
Note that the time scale in (B) has been enlarged for clarity. Curves have been obtained by numerical integration of eqns (1) to (5), with the parameter values given in Figure 1, with $InsP_3 = 0.15\ \mu M$, $C_T = 80\ \mu M$, $\alpha = 0.1$, $b = 7 \times 10^{-4}$, $V_{MP} = 4\ \mu M \cdot s^{-1}$, $K_P = 0.35\ \mu M$, $n_P = 2$. The three types of receptors are assumed to be present in the same proportions: $\lambda_1 = \lambda_2 = \lambda_3 = 0.333$.

ure 2(B), corresponding to the left-hand panel of Figure 1], because it possesses the highest affinity for $InsP_3$. This type is also more sensitive to lower Ca^{2+} concentrations than the other two types, in agreement with the steady-state behaviours shown in Figure 1. $InsP_3Rs$ exhibiting an $InsP_3$-dependent bell-shaped steady-state dependence on cytosolic Ca^{2+} [continuous line in Figure 2(B), corresponding to the middle panel of Figure 1] are always less active than the bell type, as they have a lower affinity for $InsP_3$. Moreover, one can see that the $InsP_3$-dependent bell type is less sensitive to Ca^{2+} changes than the bell type, due to the reduced level of inhibition by Ca^{2+}. Finally, with regards to the sigmoid type characterized by a much reduced inhibition by cytosolic Ca^{2+} [dotted line in Figure 2(B), corresponding to the right-hand panel of Figure 1], it is clear from the shape of the spike that the fraction of open receptors decreases because Ca^{2+} decreases, but

Figure 3 | Bifurcation diagrams showing the effect of changing the receptor density (λ^i) on the occurrence of Ca^{2+} oscillations

These diagrams show the steady-state Ca^{2+} levels as a function of the InsP$_3$ concentration. When the steady state is stable, it is indicated by a continuous line. When it is unstable, it is indicated by a dotted line; in this case, the steady state is not observed, but oscillations around this steady state occur. The maximum value of the Ca^{2+} concentration reached during the oscillations is then indicated by a dashed line. (**A** and **B**) Cells possessing a high density of one type of InsP$_3$R. The shapes of the curves are very different for the bell type (**A**) or the InsP$_3$-dependent type (**B**). When the density of receptors (λ^i) is decreased, it leads in both cases to an increase of the oscillatory domain. This increase is, however, larger for the bell type (**C**) compared with the InsP$_3$-dependent type (**D**). For further details, see the text. Results have been obtained using AUTO, as implemented by XPPAUT (Ermentrout, 2002), with the same parameters as in Figure 2. Insets show typical Ca^{2+} oscillations obtained in each case, with InsP$_3$ = 0.1 μM for (**A**) and (**C**), and 0.2 μM for (**B**) and (**D**).

Different abilities of the three receptor types to generate Ca^{2+} oscillations

It can be expected that the distinct behaviours of the three receptor types (Figure 2B) are related to different abilities to generate oscillations. Figures 3(A) and 3(B) show the bifurcation diagrams corresponding to a cell expressing the classical bell-type or the InsP$_3$-dependent bell-type receptors. In both Figures 3(A) and 3(B), the continuous line shows the steady-state Ca^{2+} level as a function of the InsP$_3$ concentration, and the dashed line shows the maximum of Ca^{2+} oscillations when they occur. Note the different scales in both diagrams. The dotted line represents the unstable steady state during oscillations; this state, which is not observed, corresponds to the Ca^{2+} concentration around which oscillations occur. The shape of the curve showing the steady-state Ca^{2+} level (stable or unstable) as a function of InsP$_3$ (continuous lines) differs between both types. For the classical bell type, this level rises, because the fraction of open receptors increases with the level of InsP$_3$ (eqn 2), for which this receptor has a high affinity (K^B = 0.2 μM). Once Ca^{2+} becomes high,

that inhibition by Ca^{2+} does not play a significant role in this diminishing phase.

inhibition by Ca^{2+} becomes predominant and the level of Ca^{2+} does not depend on the level of $InsP_3$ anymore. In contrast, for the $InsP_3$-dependent bell type (Figure 3), the steady-state level of Ca^{2+} first smoothly increases with $InsP_3$, because of the lower affinity of this type for $InsP_3$ ($K^I = 0.5$ μM). When $InsP_3$ further increases, the rise of Ca^{2+} with $InsP_3$ becomes very fast, because receptor inhibition by Ca^{2+} occurs progressively less. For both isoforms (Figures 3A–3D), oscillations occur when the (unstable) steady-state level of Ca^{2+} lies in the range of the activation and inhibition constants of the receptors ($K_{act} = 0.5$ μM and $K_{inh} = 0.247$ μM for both types); thus, one observes that oscillations occur at ~ 0.2 μM $< C_c < \sim 0.8$ μM.

We have not shown any bifurcation diagram for the sigmoid type, because, for the present choice of parameter values, this type can never display Ca^{2+} oscillations on its own. Indeed, in a closed cell (i.e. when the Ca^{2+} exchanges with the extracellular medium are not considered, as in the present model), sustained Ca^{2+} oscillations cannot occur on the basis of positive feedback of Ca^{2+} on the activity of the $InsP_3R$ only; an effective inhibitory mechanism is also required (Dupont and Goldbeter, 1993). In our simulated model of the $InsP_3R$ of the sigmoid type, the negative feedback, although present in the equations, is not strong enough (see Figure 1, right-hand panel).

The different shapes of the bifurcation curves shown in Figures 3(A) and 3(B) for the bell-type and $InsP_3$-dependent bell-type receptors have important implications when the amount of a given receptor type present in a cell is varied. Figures 3(C) and 3(D) show the effect of decreasing the amount of receptors of a given type (or, equivalently, the flux characterizing each type of channel). The oscillatory domain increases in both cases. This is due to the fact that, as there are less channels, there is less Ca^{2+} released, which postpones the inhibition for higher values of $InsP_3$. Interestingly, the increase in the oscillatory domain is much larger for the bell type than for the $InsP_3$-dependent bell type. Again, this can be explained by the fact that the steady-state level of Ca^{2+} for the bell type is much less sensitive to $InsP_3$ changes (reaching a plateau). Indeed, Ca^{2+} oscillations can only occur when the steady-state level of Ca^{2+} lies in the range of the activation and inhibition constants of the receptors, which favours the

existence of a large domain for $InsP_3$, leading to Ca^{2+} oscillations (the value of the steady Ca^{2+} level at which oscillations disappear is approximately the same for both types). Another noticeable difference between both types is that for the $InsP_3$-dependent type (Figures 3B and 3D), the oscillatory domain is shifted towards higher $InsP_3$ concentrations when decreasing the amount of receptor, which is not the case for the other type (Figures 3A and 3C). If λ becomes too small, oscillations are no longer possible whatever the $InsP_3$ concentration, because there is not enough Ca^{2+} released (data not shown). Thus for both receptor types, the size of the oscillatory domain first increases and later decreases when decreasing λ (data not shown).
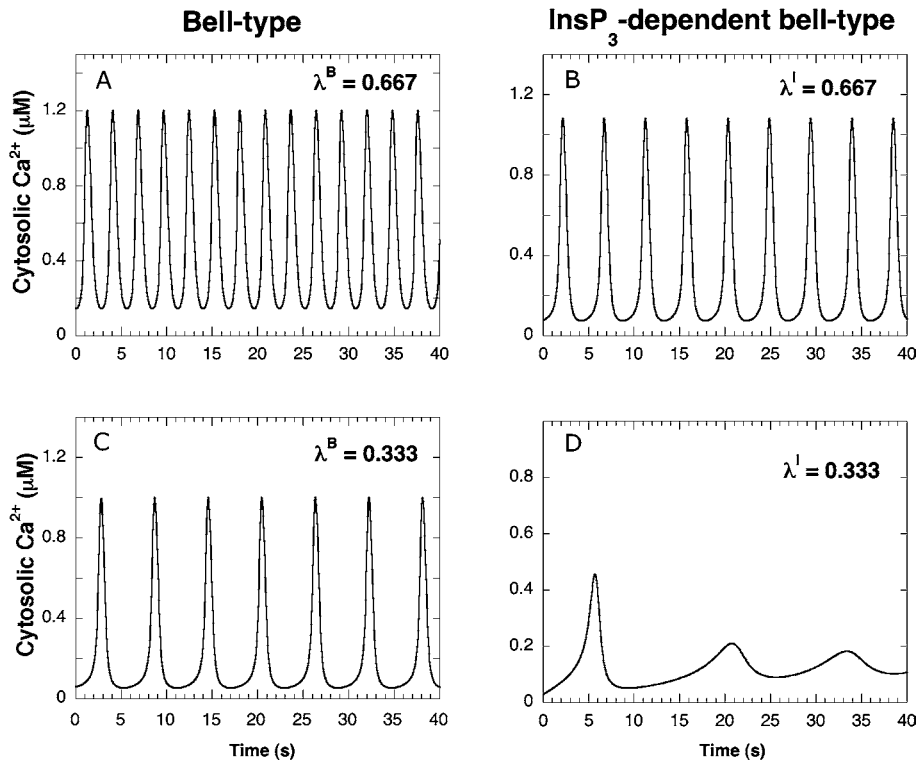
Figure 3, in fact, indicates that $InsP_3Rs$ exhibiting various steady-state open-probability curves will display different robustness with respect to changes in their expression levels in a cell. As an illustration, Figure 4 shows the effect on Ca^{2+} oscillations of decreasing the amount of $InsP_3Rs$ either of the bell type (Figures 4A and 4C) or of the $InsP_3$-dependent bell type (Figures 4B and 4D). All the other parameters (including the $InsP_3$ level) are kept constant. For the bell type, Ca^{2+} oscillations persist when reducing the amount of channel by half. The period and amplitude of oscillations, however, both decrease with the channel density (Figure 4C). Oscillations due to the $InsP_3$-dependent bell type are, in contrast, transformed into damped oscillations when reducing the amount of channels by half (Figure 4D); this results from the fact that the $InsP_3$ concentration is now below the threshold inducing oscillations, due to the shift of the oscillatory domain when decreasing the receptor amount, $λ^I$ (see Figures 3B and 3D). Sustained oscillations can be recovered by increasing $InsP_3$. However, on average, the probability of obtaining sustained oscillations when decreasing the amount of receptors of the $InsP_3$-dependent bell type is weaker than for the bell type, given that the oscillatory domain is smaller.

Dual effect of the sigmoid-type InsP₃R on Ca²⁺ oscillations

We have seen that the receptor of the sigmoid type, for which inhibition at high Ca^{2+} is not significant, behaves very differently from the other receptor types, as it cannot generate Ca^{2+} oscillations. In contrast, activation of this receptor type provides a constant Ca^{2+}

Figure 4 | Temporal evolution of Ca^{2+} at different receptor densities

Decreasing the amount of receptor by a factor of 2 does not affect the existence of Ca^{2+} oscillations if these receptors are of the bell type (**A**, **C**), but transforms sustained oscillations into damped ones if these receptors are of the 'InsP$_3$-dependent bell type' (**B**, **D**). All equations and parameters are the same as decribed in Figure 3.

influx into the cytosol. Although this influx always remains rather weak (as this receptor has a low affinity for InsP$_3$), it can, however, have a major effect on the oscillations induced by both other types. Figure 5 shows the bifurcation diagram of a system characterized by 50% of the bell-type receptor and 50% of the sigmoid type. Thus, by comparing Figures 3(C) and 5(A), and Figures 3(D) and 5(B), one can directly see the effect of the sigmoid type on both other types. Clearly, the oscillatory domain is much reduced in both cases. The flux of Ca^{2+} through the non-inhibited InsP$_3$Rs, indeed, increases the Ca^{2+} level for all values of InsP$_3$ concentrations, without providing the feedback necessary for oscillations to occur. Interestingly, the bifurcation diagrams for the bell type and the InsP$_3$-dependent bell type (Figures 5A and 5B) become very similar when mixed to the sigmoid type. In both cases, the rapid increase of the steady-state level of Ca^{2+} with InsP$_3$, indeed, becomes predominant. Thus, as shown in Figures 6(A) and 6(C), the addition of InsP$_3$Rs of the sigmoid type tends to suppress Ca^{2+} oscillations generated by the same amount of the bell-type receptor. Interestingly, the oscillations shown in Figure 6(A) are maintained if InsP$_3$Rs of the InsP$_3$-dependent bell type ($\lambda^I = 0.5$) are added (data not shown).

Although in the situations encountered above, the receptors of the sigmoid type have an inhibitory effect on Ca^{2+} oscillations, simulations predict that this is not always the case. Figure 6(B) shows damped oscillations obtained with a low density of bell-type and InsP$_3$-dependent bell-type receptors. In this case, oscillations cannot be sustained, because the global flux of Ca^{2+} is too small to reach the typical Ca^{2+} concentration in which activation/inhibition by Ca^{2+} may occur. Sustained Ca^{2+} oscillations can be obtained in this case by the addition of InsP$_3$Rs of the sigmoid type, which increases the total Ca^{2+} release from the ER. It is interesting to note that when starting from a situation such as that shown in

These curves have to be compared with Figures 3(C) and 3(D) respectively. In both cases, the oscillatory domain is much reduced. Results have been obtained as described in Figure 3.
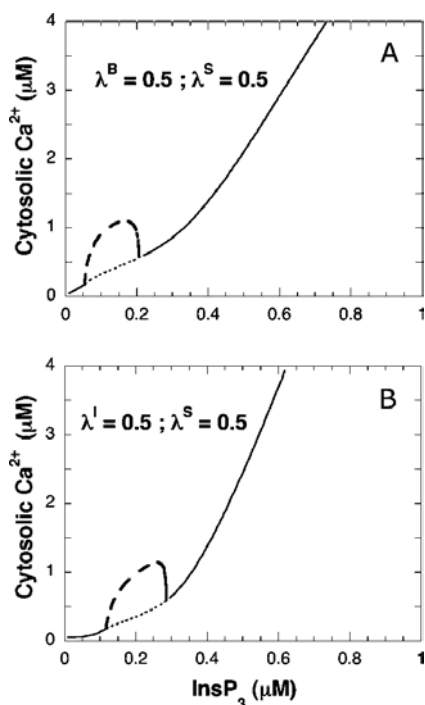

by different subpopulations of receptors or artificially expressing one or the other isoform. A modelling approach can thus help to make the link between these various data and suggest some conclusions about the respective role of each isoform.

We have classified the possible behaviours that have been reported in the literature for the InsP$_3$Rs as follows.

(i) The classical bell-type InsP$_3$R that is activated by low cytosolic Ca^{2+} and inhibited by higher concentrations of this compound, with no interplay between InsP$_3$ and Ca^{2+} in channel regulation.
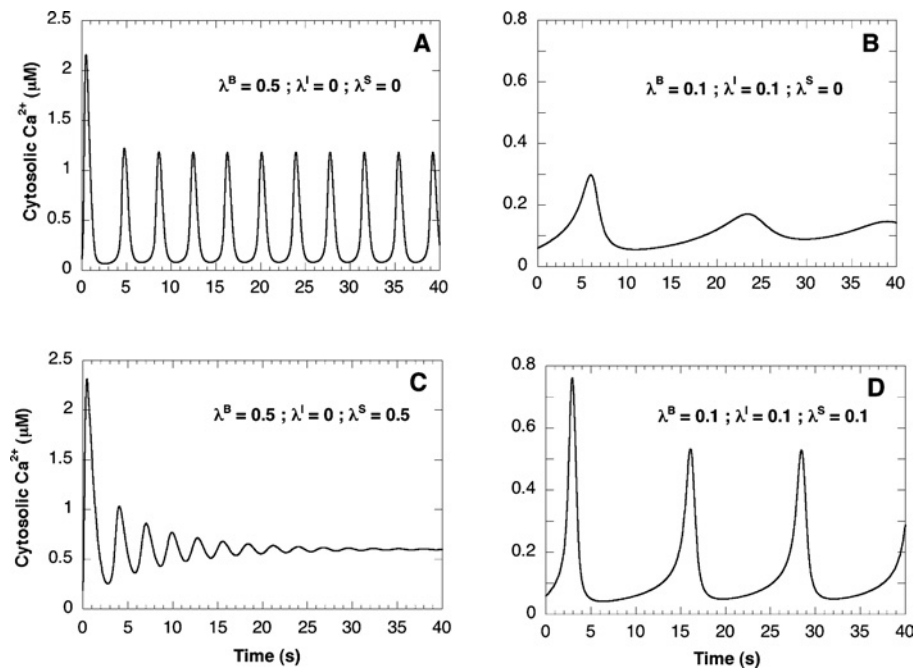
(ii) The InsP$_3$-dependent bell type which behaves as the bell type, except that the threshold for channel inhibition by Ca^{2+} increases with InsP$_3$. This interaction between InsP$_3$ and Ca^{2+} is responsible for the shift of the bell-shaped curve to the right when increasing Ca^{2+}.

(iii) The sigmoid type which behaves as the InsP$_3$-dependent bell type, except that the threshold for channel inhibition by Ca^{2+} lies outside the physiological range reached during oscillations (in this global approach, we do not consider the high Ca^{2+} concentrations experienced at the mouth of the channel). This type, for which experimental data are most controversial, however, deserves some theoretical investigation, because it has been reported to have a drastic effect on Ca^{2+} oscillations (Miyakawa et al., 1999).

Despite these rather modest differences, modelling predicts that these receptors will play different roles in the control of the intracellular Ca^{2+} dynamics. The first major difference is that the receptor type for which Ca^{2+}-induced inhibition plays a minor role cannot generate Ca^{2+} oscillations on its own. In fact, this conclusion is similar to the results obtained with the Ca^{2+}-induced Ca^{2+}-release models of the 'first generation' (Dupont and Goldbeter, 1993) where Ca^{2+} oscillations required some Ca^{2+} exchange with the extracellular medium. Interestingly, it has been shown that BCR stimulation of DT40 cells elicits Ca^{2+} entry from the external medium in a manner that is dependent on the expression of the InsP$_3$R3 isoform, which is the most probable one to display a sigmoid type of behaviour (Morita et al., 2004; Guillemette et al., 2005). One could thus speculate that the isoform showing the smallest level of inhibition does usually not provide an oscillatory unit by itself, but is rather related to Ca^{2+} entry.

Figure 6(D) (i.e. where the overall receptor density is low), the suppression of any type of receptors provokes the disappearance of Ca^{2+} oscillations. In the same manner, for slightly higher values of receptor densities, one can get situations where a combination of two out of the three isoforms are enough to produce oscillations, whatever the nature of these receptors [for example, for $\lambda^B = 0.25$, $\lambda^I = 0.35$ and $\lambda^S = 0.15$ in the same conditions as in Figure 6(D); data not shown].

Discussion

The detailed investigation of the effect of specific InsP$_3$ receptor isoforms on cellular Ca^{2+} signals is a complex matter. Its full understanding involves electrophysiological and biochemical characterizations of the three isoforms, as well as the detailed analysis of Ca^{2+} signals in cells either characterized

Figure 6 | Addition of InsP₃Rs of the sigmoid type can either abolish (A, C) or restore (B, D) oscillations in a simulated cell, depending on the overall population of InsP₃Rs

(**A**) Sustained Ca^{2+} oscillations obtained in a cell supposed to have InsP₃Rs of the 'bell type' only. (**B**) When adding the same amount of receptors of the 'sigmoid type', oscillations disappear. (**C**) Damped Ca^{2+} oscillations obtained in a cell characterized by a low density of receptors. (**D**) When adding a small amount of receptors of the 'sigmoid type' to the same simulated cell, oscillations become sustained (see the text). Results were obtained as described for Figure 4, except for the values of the λ^i, which are indicated, and for InsP₃ = 0.22 μM (**A, C**) or 0.35 μM (**B, D**).

In contrast, the bell type and the InsP₃-dependent bell type both display Ca^{2+} oscillations for a large range of parameter values. These oscillations display very similar shapes. However, the steady-state Ca^{2+} concentration is very sensitive to InsP₃ for the InsP₃-dependent bell-type receptors. For the classical bell type in contrast, the steady-state Ca^{2+} level rapidly reaches a plateau value when increasing the InsP₃ concentration (Figure 3A). Interestingly, the model also predicts that these receptors behave quite differently when changing their density inside the cell. Although in both cases, these receptors display Ca^{2+} oscillations at a larger range of InsP₃ concentrations when they are present in smaller amounts, this increase in the oscillatory domain is much larger for the classical bell-type receptor (Figures 3C and 3D). Moreover, for the latter receptor type, the minimal InsP₃ concentration necessary to get oscillations remains practically unchanged, while this concentration increases when decreasing the receptor density for the InsP₃-dependent bell type. On the whole, at low receptor density, the classical bell-type receptor is thus more susceptible to generate Ca^{2+} oscillations.

To make an hypothetical link with the experimental results, one can speculate that the above defined bell-type receptor corresponds to the type 2 InsP₃R, the InsP₃-dependent bell type to the type 1 and the sigmoid type to the type 3. In this framework, our theoretical results match with the observation that mutant DT40 cells expressing only InsP₃R1 isoforms show rapidly damped oscillations, whereas mutant cells expressing InsP₃R2 show very regular and robust Ca^{2+} oscillations (Miyakawa et al., 1999). Our theoretical results shown in Figures 5(A), 5(B), 6(A) and 6(C) could also match with the observation performed in HeLa and COS cells that InsP₃R3 tend to inhibit Ca^{2+} oscillations (Hattori et al., 2004).

The model also suggests that the role of a given isoform in favouring or not favouring Ca^{2+}

oscillations may be dependent on other factors, such as the density of the other receptor types. Simulations, indeed, show that when the overall density of InsP$_3$Rs in a cell is too low for sustained Ca^{2+} oscillations to occur, addition of a small amount of the sigmoid type of InsP$_3$Rs can boost up Ca^{2+} oscillations, exactly as the combination of a subthreshold stimulation and Ca^{2+} entry can provoke oscillations (Rooney et al., 1991). These theoretical conclusions thus first suggest that experimental results obtained in HeLa or COS cells (Miyakawa et al., 1999) might not apply to all cell types, and that the InsP$_3$R3 does not always behave as an 'anti-oscillatory unit'. Second, these preliminary simulations also suggest that the total density of InsP$_3$Rs plays a crucial role in determining the global Ca^{2+} dynamics. It should be interesting to model changes in receptor density occuring in physiological conditions, as, for example, during chronic agonist stimulation.

In the future, this approach needs to be pursued to investigate the spatial aspects related to the spatially inhomogeneous distribution of receptor subtypes. It is clear, indeed, that the functional interactions between the three subtypes will be affected by their possible spatial clustering. The regulations by accessory proteins, which clearly differ from one InsP$_3$R isoform to the other, is another point that deserves to be further explored and that might help to improve our understanding of the origin of the impressive variety of InsP$_3$-mediated intracellular Ca^{2+} signals.

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