

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: $\text{InsP}_3\text{R}1$, $\text{InsP}_3\text{R}2$ and $\text{InsP}_3\text{R}3$. In all cell types, functional channels result from the

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Abbreviations used: InsP_3 , inositol 1,4,5-trisphosphate; InsP_3R , InsP_3 receptor; ER, endoplasmic reticulum.

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_3R 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_3Rs affect the time course of cytosolic Ca^{2+} concentration after agonist stimulation. Thus in DT40 B-cells expressing a single InsP_3R 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 $\text{InsP}_3\text{R}2$ 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 $\text{InsP}_3\text{R}1$ or $\text{InsP}_3\text{R}3$ were knocked down by RNAi (RNA interference) in HeLa and COS-7 cells. In both cell types, knockdown of $\text{InsP}_3\text{R}1$ tends to decrease the number of Ca^{2+} spikes, whereas knockdown of $\text{InsP}_3\text{R}3$ 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_3Rs 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_3Rs 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 $\text{InsP}_3\text{R}1$ to $\text{InsP}_3\text{R}3$ 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_3R 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 $\text{InsP}_3\text{R}3$ 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 $\text{InsP}_3\text{R}2$ has also been considered in the case of rat adrenal chromaffin cells (Inoue et al., 2003).

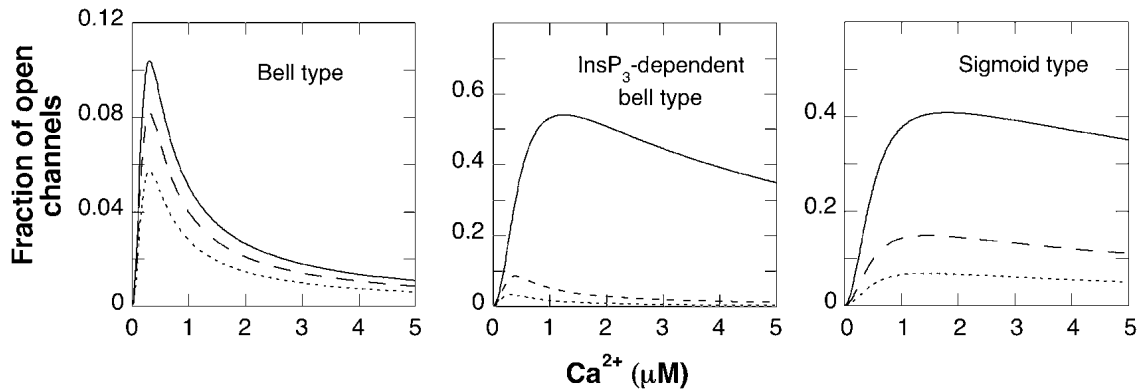
In the present work, we have theoretically studied the possible implications of the different regulations by InsP_3 and Ca^{2+} of the various InsP_3 isoforms on the global Ca^{2+} dynamics. To this end, we first develop phenomenological models accounting for the distinct steady-state behaviours that 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_3R 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_3Rs into a model for the dynamics of cellular Ca^{2+} signalling. We find that slight differences in the Ca^{2+} regulatory properties of these $\text{InsP}_3\text{Rs}/\text{Ca}^{2+}$ 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_3R isoforms.

Mathematical model

The steady-state open probability of the $\text{InsP}_3/\text{Ca}^{2+}$ 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 InsP_3Rs as a function of the concentration of Ca^{2+} at the cytosolic side of the channel, for three concentrations of InsP_3 : 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: $k_+^B = 14.4606 \mu\text{M}^{-3} \cdot \text{s}^{-1}$, $k_-^B = 0.217 \text{s}^{-1}$, $K_{\text{act}} = 0.5 \mu\text{M}$, $K_B = 0.2 \mu\text{M}$, $n_i = 3$, $n = 3$, $n_a = 2$. The inhibition constant of the receptor by Ca^{2+} was calculated by $K_{\text{inh}}^B = (k_-^B/k_+^B)^{1/n_i} = 0.247 \mu\text{M}$. InsP_3 -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: $K_i^1 = K^1 = 0.5 \mu\text{M}$. Sigmoid type: curves have been obtained by solving eqns (1) and (2) at steady state, with the same parameter values as for the InsP_3 -dependent type, except for: $k_-^S = 21.7 \text{s}^{-1}$, $K_i^S = K^S = 4 \mu\text{M}$. Thus $K_{\text{inh}}^S = 1.145 \mu\text{M}$.



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 Ca^{2+} -releasing activity of the InsP_3R by Ca^{2+} , with apparently no interplay between the regulations by Ca^{2+} and by InsP_3 (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 InsP_3R of the bell type that has been inhibited by Ca^{2+} , is given by:

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

where k_+^B is the rate of inhibition of this InsP_3R type by cytosolic Ca^{2+} and k_-^B is the rate of relief from this inhibition. Activation of the receptor by Ca^{2+} 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 Ca^{2+} . 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:

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

where K^B is the half-saturation constant of the bell-type InsP_3R for InsP_3 (see below for the value of this parameter). The InsP_3 dependence of Ca^{2+} release is proposed to be non-cooperative. The fraction of open InsP_3R of this bell type at steady state as a function of Ca^{2+} concentration is shown in Figure 1 (left-hand panel) for three values of the InsP_3 concentration. As expected from eqn (2), an increase in InsP_3 simply shifts the bell-shaped curve towards higher opening levels (but not horizontally).

(ii) In other experiments, an interplay between the regulations by Ca^{2+} and InsP_3 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 $\text{InsP}_3\text{R}/\text{Ca}^{2+}$ 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:

$$\frac{dR_i^I}{dt} = k_+^I (1 - R_i^I) \cdot \frac{C_c^{n_i}}{1 + \left(\frac{C_c}{K_{\text{act}}}\right)^{n_a}} \cdot \frac{(K_i^I)^n}{(K_i^I)^n + (\text{InsP}_3)^n} - k_-^I R_i^I \quad (3)$$

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:

$$IR_a^i = (1 - R_i^I) \cdot \frac{\text{InsP}_3}{K^I + \text{InsP}_3} \cdot \frac{C_a^{n_a}}{C_a^{n_a} + K_{\text{act}}^{n_a}} \quad (4)$$

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 of 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 $K^B = 0.2 \mu\text{M}$, $K^I = 0.5 \mu\text{M}$ and $K^S = 4 \mu\text{M}$ 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:

$$\frac{dC_c}{dt} = \sum_{i=1}^3 \lambda^i (b + IR^i) \cdot [C_T - C_c(\alpha + 1)] - V_{\text{MP}} \frac{C_c^{n_p}}{C_c^{n_p} + K_p^{n_p}} \quad (5)$$

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.

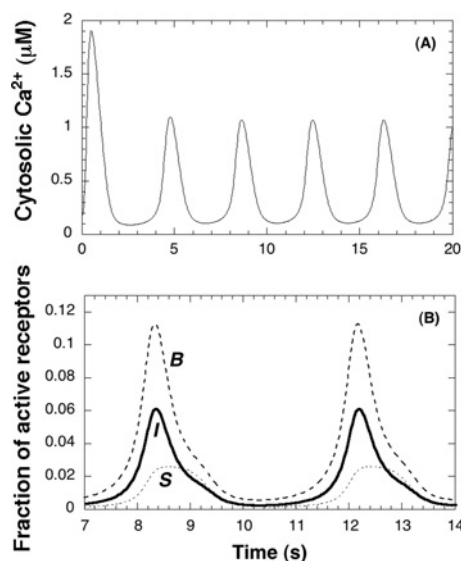
Results

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

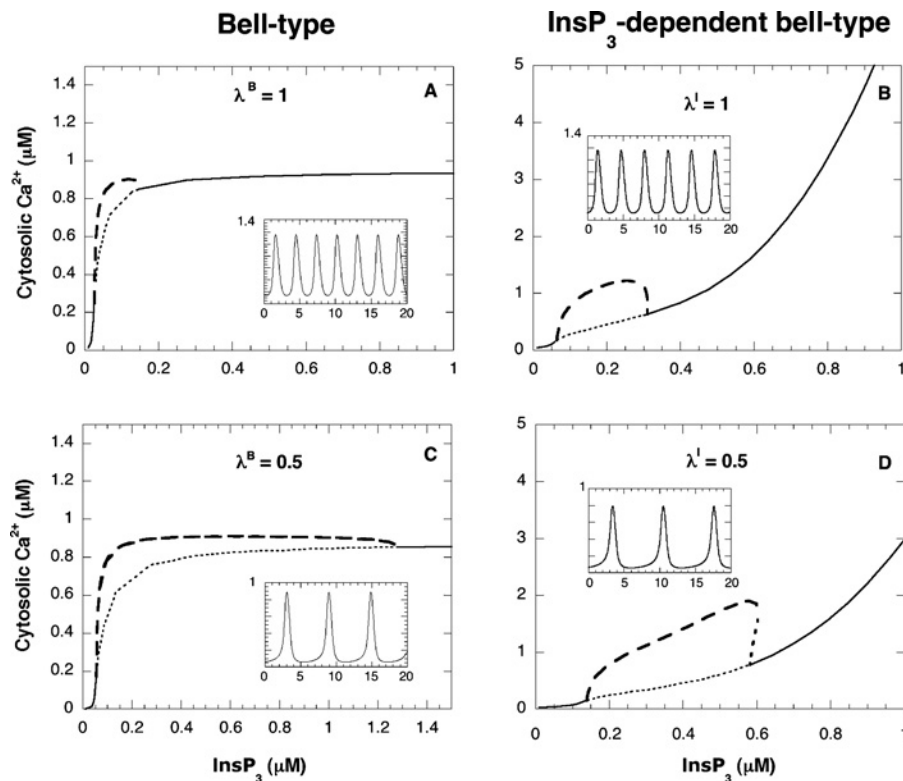
Note that the time scale in (B) has been enlarged for clarity. Curves have been obtained by numerical integration of eqns (1) to (5), with the parameter values given in Figure 1, with $\text{InsP}_3 = 0.15 \mu\text{M}$, $C_T = 80 \mu\text{M}$, $\alpha = 0.1$, $b = 7 \times 10^{-4}$, $V_{\text{MP}} = 4 \mu\text{M} \cdot \text{s}^{-1}$, $K_p = 0.35 \mu\text{M}$, $n_p = 2$. 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_3R . 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 $\text{InsP}_3 = 0.1 \mu\text{M}$ for **(A)** and **(C)**, and $0.2 \mu\text{M}$ for **(B)** and **(D)**.



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

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 ($K^B = 0.2 \mu\text{M}$). Once Ca^{2+} becomes high,

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 ($K^I = 0.5 \mu\text{M}$). 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 ($K_{\text{act}} = 0.5 \mu\text{M}$ and $K_{\text{inh}} = 0.247 \mu\text{M}$ for both types); thus, one observes that oscillations occur at $\sim 0.2 \mu\text{M} < C_c < \sim 0.8 \mu\text{M}$.

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).

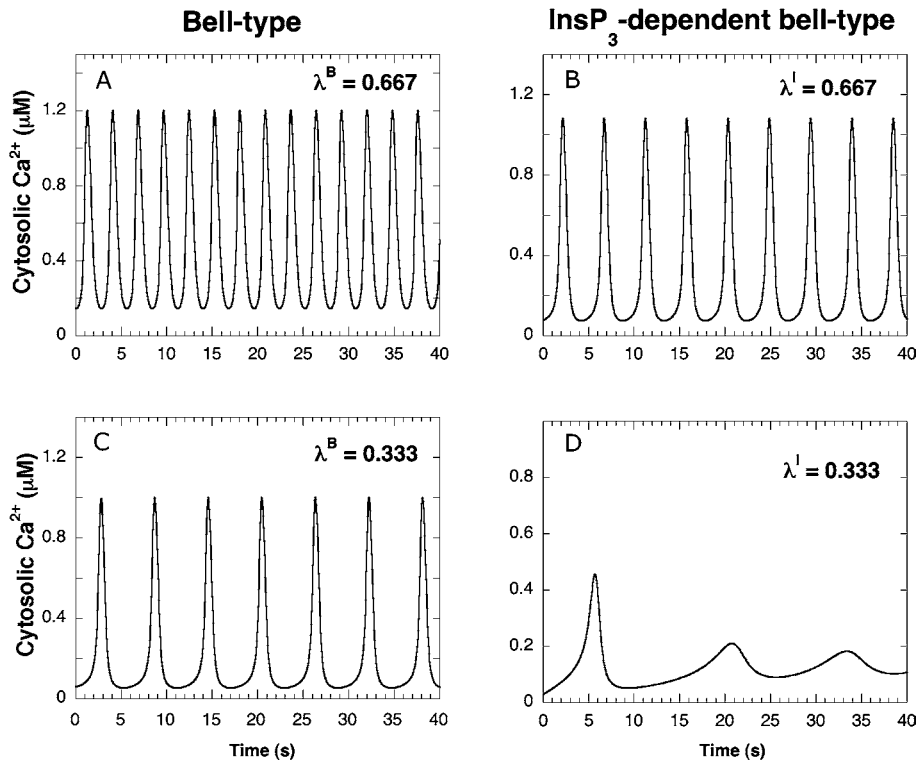
Figure 3, in fact, indicates that InsP_3Rs exhibiting various steady-state open-probability curves will display different robustness with respect to changes in their expression levels in a cell. As an illustration, Figure 4 shows the effect on Ca^{2+} oscillations of decreasing the amount of InsP_3Rs 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 InsP_3R on Ca^{2+} 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 'InsP₃-dependent bell type' (B, D). All equations and parameters are the same as described in Figure 3.



influx into the cytosol. Although this influx always remains rather weak (as this receptor has a low affinity for InsP₃), 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 InsP₃Rs, indeed, increases the Ca^{2+} level for all values of InsP₃ concentrations, without providing the feedback necessary for oscillations to occur. Interestingly, the bifurcation diagrams for the bell type and the InsP₃-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 InsP₃, indeed, becomes predominant. Thus, as shown in Fig-

ures 6(A) and 6(C), the addition of InsP₃Rs 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 InsP₃Rs of the InsP₃-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 InsP₃-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 InsP₃Rs 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

Figure 5 | Bifurcation diagrams showing the effect of adding receptors of the sigmoid type to an homogeneous population of receptors of the bell type (A) or of the InsP_3 -dependent type (B)

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.

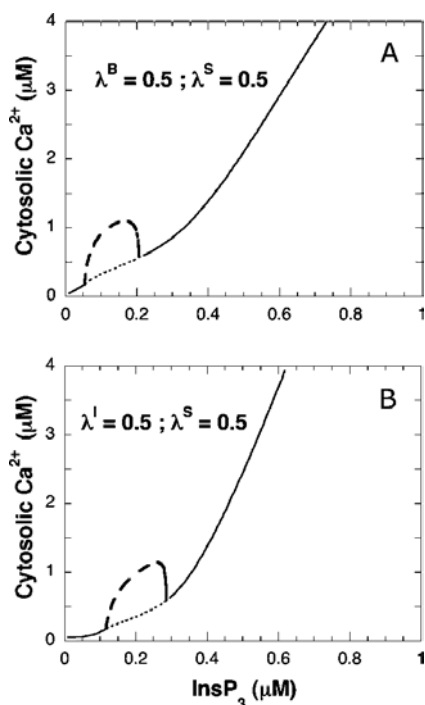


Figure 6(D) (i.e. where the overall receptor density is low), the suppression of any type of receptors provokes the disappearance of Ca^{2+} oscillations. In the same manner, for slightly higher values of receptor densities, one can get situations where a combination of two out of the three isoforms 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

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_3Rs as follows.

(i) The classical bell-type InsP_3R 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.

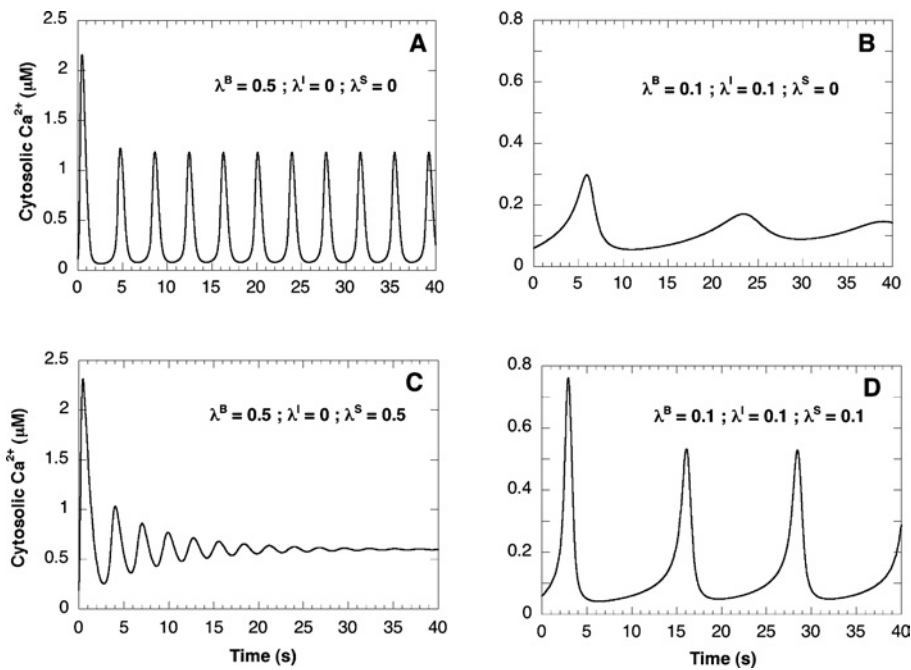
(ii) The InsP_3 -dependent bell type which behaves as the bell type, except that the threshold for channel inhibition by Ca^{2+} increases with InsP_3 . This interaction between InsP_3 and Ca^{2+} is responsible for the shift of the bell-shaped curve to the right when increasing Ca^{2+} .

(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 $\text{InsP}_3\text{R}3$ 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 | Addition of InsP_3Rs of the sigmoid type can either abolish (A, C) or restore (B, D) oscillations in a simulated cell, depending on the overall population of InsP_3Rs

(A) Sustained Ca^{2+} oscillations obtained in a cell supposed to have InsP_3Rs 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 $\text{InsP}_3 = 0.22 \mu\text{M}$ (A, C) or $0.35 \mu\text{M}$ (B, D).



In contrast, the bell type and the InsP_3 -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 InsP_3 for the InsP_3 -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 InsP_3 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 InsP_3 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 InsP_3 concentration necessary to get oscillations remains practically unchanged, while this concentration increases when decreasing the receptor den-

sity for the InsP_3 -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 InsP_3R , the InsP_3 -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 $\text{InsP}_3\text{R1}$ isoforms show rapidly damped oscillations, whereas mutant cells expressing $\text{InsP}_3\text{R2}$ 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 $\text{InsP}_3\text{R3}$ 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 InsP₃Rs in a cell is too low for sustained Ca²⁺ oscillations to occur, addition of a small amount of the sigmoid type of InsP₃Rs can boost up Ca²⁺ oscillations, exactly as the combination of a subthreshold stimulation and Ca²⁺ 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 InsP₃R3 does not always behave as an ‘anti-oscillatory unit’. Second, these preliminary simulations also suggest that the total density of InsP₃Rs plays a crucial role in determining the global Ca²⁺ dynamics. It should be interesting to model changes in receptor density occurring 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 InsP₃R 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 InsP₃-mediated intracellular Ca²⁺ signals.

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References

Berridge, M.J., Lipp, P. and Bootman, M.D (2000) The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell Biol.* **1**, 11–21

Bezprozvanny, I., Watras, J. and Ehrlich, B. (1991) Bell-shaped calcium-response curves of Ins(1,4,5)P₃- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature (London)* **351**, 751–754

Champeil, P., Combettes, L., Berthon, B., Doucet, E., Orlowski, S. and Claret, M. (1989) Fast kinetics of calcium release induced by myo-inositol trisphosphate in permeabilized rat hepatocytes. *J. Biol. Chem.* **264**, 17665–17673

Combettes, L., Dupont, G. and Parys, J. (2004) New mechanisms and functions in Ca²⁺ signalling. *Biol. Cell* **96**, 1–2

De Smedt, H., Missiaen, L., Parys, J., Henning R., Sienaert, I., Vanlingen, S., Gijsens, A., Himpens, B. and Casteels, R. (1997) Isoform diversity of the inositol trisphosphate receptor in cell types of mouse origin. *Biochem. J.* **322**, 575–583

Dufour, J.F., Arias, I.M. and Turner, T.J. (1997) Inositol 1,4,5-trisphosphate and calcium regulate the calcium channel function of the hepatic inositol 1,4,5-trisphosphate receptor. *J. Biol. Chem.* **272**, 2675–2681

Dupont, G. and Goldbeter, A. (1993) One-pool model for Ca²⁺ oscillations involving Ca²⁺ and inositol 1,4,5-trisphosphate as co-agonists for Ca²⁺ release. *Cell Calcium* **14**, 311–322

Dupont, G. and Swillens, S. (1996) Quantal release, incremental detection and long-period Ca²⁺ oscillations in a model based on regulatory Ca²⁺-binding sites along the permeation pathway. *Biophys. J.* **71**, 1714–1722

Dyer, J. and Michelangeli, F. (2001) Inositol 1,4,5-trisphosphate receptor isoforms show similar Ca²⁺ release kinetics. *Cell Calcium* **30**, 245–250

Ermentrout, B. (2002) Solving and analyzing dynamical systems using XPPAUT. In *Computational Cell Biology* (Fall, C., Marland, E., Wagner, J. and Tyson, J., eds.), Springer, New York

Finch, E., Turner, T. and Goldin, S. (1991) Calcium as coagonist of inositol 1,4,5-trisphosphate concentrations during calcium release. *Science (Washington, D.C.)* **252**, 443–446

Guillemette, J., Caron, A., Regimbald-Dumas, Y., Arguin, G., Mignery, G., Boulay, G. and Guillemette, G. (2005) Expression of a truncated form of inositol 1,4,5-trisphosphate receptor type III in the cytosol of DT40 triple inositol 1,4,5-trisphosphate receptor-knockout cells. *Cell Calcium* **37**, 97–104

Haberichter, T., Roux, E., Marhl, M. and Mazat, J.-P. (2002) The influence of different InsP₃ receptor isoforms on Ca²⁺ signaling in tracheal smooth muscle cells. *Bioelectrochemistry* **57**, 129–138

Hagar, R., Burgstahler, A., Nathanson, M. and Ehrlich, B. (1998) Type III InsP₃ receptor channel stays open in the presence of increased calcium. *Nature (London)* **396**, 81–84

Hattori, M., Suzuki, A., Higo, T., Miyauchi, H., Michikawa, T., Nakamura, T., Inoue, T. and Mikoshiba, K. (2004) Distinct roles of inositol 1,4,5-trisphosphate receptor types 1 and 3 in Ca²⁺ signalling. *J. Biol. Chem.* **279**, 11967–11975

Inoue, M., Iin, H., Imagana, I., Ogawa, K. and Warashina, A. (2003) InsP₃ receptor type 2 and oscillatory and monophasic Ca²⁺ transients in rat adrenal chromaffin cells. *Cell Calcium* **35**, 59–70

Kaftan, E., Ehrlich, B. and Watras, J. (1997) Inositol 1,4,5-trisphosphate (InsP₃) and calcium interact to increase the dynamic range of InsP₃ receptor-dependent calcium signaling. *J. Gen. Physiol.* **110**, 529–538

LeBeau, A., Yule, D., Groblewski, G. and Sneyd, J. (1999) Agonist-dependent phosphorylation of the inositol 1,4,5-trisphosphate receptor. *J. Gen. Physiol.* **113**, 851–871

Mak, D.-O., McBride, S. and Foskett, J.K. (1998) Inositol 1,4,5-trisphosphate activation of inositol trisphosphate receptor Ca²⁺ channel by ligand tuning of Ca²⁺ inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 15821–15825

Mak, D.-O., McBride, S. and Foskett, J.K. (2001a) ATP regulation of recombinant type 3 inositol 1,4,5-trisphosphate receptor gating. *J. Gen. Physiol.* **117**, 447–456

Mak, D.-O., McBride, S. and Foskett, J.K. (2001b) Regulation by Ca²⁺ and inositol 1,4,5-trisphosphate (InsP₃) of single recombinant type 3 InsP₃ receptor channels: Ca²⁺ activation uniquely distinguishes types 1 and 3 InsP₃ receptors. *J. Gen. Physiol.* **117**, 435–446

Missiaen, L., Parys, J., Sienaert, I., Maes, K., Kunzelmann, K., Takahashi, M., Tanzawa, K. and De Smedt, H. (1998) Functional properties of the type-3 InsP₃ receptor in 16HBE14o– bronchial mucosal cells. *J. Biol. Chem.* **273**, 8983–8986

- Miyakawa, T., Maeda, A., Yamazawa, T., Hirose, K., Kurosaki, T. and Iino, M. (1999) Encoding of Ca^{2+} signals by differential expression of IP_3 receptor subtypes. *EMBO J.* **18**, 1303–1308
- Morel, J.-L., Fritz, N., Lavie, J.-L. and Mironneau, J. (2003) Crucial role of type 2 inositol 1,4,5-trisphosphate receptors for acetylcholine-induced Ca^{2+} oscillations in vascular myocytes. *Arterioscler. Thromb. Vasc. Biol.* **23**, 1567–1575
- Morita, T., Tanimura, A., Nezu, A., Kurosaki, T. and Tojyo, Y. (2004) Functional analysis of the green fluorescent protein-tagged inositol 1,4,5-trisphosphate receptor type 3 in Ca^{2+} release and entry in DT40 B-lymphocytes. *Biochem. J.* **382**, 793–801
- Moraru, I., Kaftan, E., Ehrlich, B. and Watras, J. (1999) Regulation of type 1 inositol 1,4,5-trisphosphate-gated calcium channels by InsP_3 and calcium. *J. Gen. Physiol.* **113**, 837–849
- Patterson, R., Boehning, D. and Snyder, S. (2004) Inositol 1,4,5-trisphosphate receptors as signal integrators. *Annu. Rev. Biochem.* **73**, 437–465
- Ramos-Franco, J., Fill, M. and Mignery, G. (1998) Isoform-specific function of single inositol 1,4,5-trisphosphate receptor channels. *Biophys. J.* **75**, 834–839
- Rooney, T., Renard, D., Sass, E. and Thomas, A. (1991) Oscillatory cytosolic Ca^{2+} waves independent of stimulated inositol 1,4,5-trisphosphate formation in hepatocytes. *J. Biol. Chem.* **266**, 12272–12282
- Schuster, S., Marhl, M. and Hofer, T. (2002) Modelling of simple and complex calcium oscillations. From single-cell responses to intercellular signalling. *Eur. J. Biochem.* **269**, 1333–1355
- Sneyd, J. and Dufour, J. (2002) A dynamic model of the type-2 inositol trisphosphate receptor. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 2398–2403
- Taylor, C. and Laude, A. (2002) IP_3 receptors and their regulation by calmodulin and cytosolic Ca^{2+} . *Cell Calcium* **32**, 321–334
- Taylor, C., Genazzani, A. and Morris, S. (1999) Expression of inositol trisphosphate receptors. *Cell Calcium* **26**, 237–251
- Vermassen, E., Parys, J.B. and Mauger, J.-P. (2004) Subcellular distribution of the inositol 1,4,5-trisphosphate receptors: functional relevance and molecular determinants. *Biol. Cell* **96**, 3–18
- Wojcikiewicz, R. and Luo, S. (1998) Differences among type I, II, and III inositol-1,4,5-trisphosphate receptors in ligand-binding affinity influence the sensitivity of calcium stores to inositol-1,4,5-trisphosphate. *Mol. Pharmacol.* **53**, 656–662
- Yule, D., Straub, S. and Bruce, J. (2003) Modulation of Ca^{2+} oscillations by phosphorylation of $\text{Ins}(1,4,5)\text{P}_3$ receptors. *Biochem. Soc. Trans.* **31**, 954–957

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