Kinetic model of the inositol trisphosphate receptor that shows both steady-state and quantal patterns of Ca^{2+} release from intracellular stores

Alan P. DAWSON*[1], Edward J. A. LEA* and Robin F. IRVINE†

*School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K., and †Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, U.K.

The release of Ca^{2+} from intracellular stores via $InsP_3$ receptors shows anomalous kinetics. Successive additions of low concentrations of $InsP_3$ cause successive rapid transients of Ca^{2+} release. These quantal responses have been ascribed to all-or-none release from stores with differing sensitivities to $InsP_3$ or, alternatively, to a steady-state mechanism where complex kinetic properties of the $InsP_3$ receptor allow partial emptying of all the stores. We present here an adaptive model of the $InsP_3$ receptor that can show either pattern, depending on the imposed experimental conditions. The model proposes two interconvertible conformational states of the receptor: one state binds $InsP_3$ rapidly, but with low affinity, whereas the other state binds slowly, but with high affinity. The model shows repetitive increments of Ca^{2+} release in the absence of a Ca^{2+} gradient, but more pronounced incremental behaviour when released Ca^{2+} builds up at the mouth of the channel. The sensitivity to $InsP_3$ is critically dependent on the density of $InsP_3$ receptors, so that different stores can respond to different concentration ranges of $InsP_3$. Since the model generates very high Hill coefficients ($h \approx 7$), it allows all-or-none release of Ca^{2+} from stores of differing receptor density, but questions the validity of the use of h values as a guide to the number of $InsP_3$ molecules needed to open the channel. The model presents a mechanism for terminating Ca^{2+} release in the presence of positive feedback from released Ca^{2+}, thereby providing an explanation of why elementary Ca^{2+} signals ('blips' and 'puffs') do not inevitably turn into regenerative waves.

Key words: blip, channel, Hill number, increment detection, $InsP_3$ receptor, puff.

INTRODUCTION

It has been recognized for many years that the release of Ca^{2+} from intracellular stores via activation of $InsP_3$ receptors has some very unusual properties. A very large number of papers over the years have reported that submaximal doses of $InsP_3$ rapidly release a part of the stored Ca^{2+}, and further increments of $InsP_3$ cause further transients of Ca^{2+} release. The term 'quantal Ca^{2+} release' was introduced by Muallem et al. [1], but the phenomenon has also been described as 'increment detection' by Meyer and Stryer [2]. Since that time, there have been two competing explanations for quantal release. The first is that cells contain a series of Ca^{2+} stores of various sensitivities to $InsP_3$, such that any given $InsP_3$ dose will empty a particular set of stores and leave the others intact (a truly quantal or all-or-none model). The second is that $InsP_3$ receptors, because of their kinetic properties, can adapt to a particular $InsP_3$ concentration, such that a given $InsP_3$ concentration can partially empty all of the stores. Raising the $InsP_3$ concentration can re-activate the receptors to allow more Ca^{2+} release. Since, in the intact system, partial emptying of the stores must entail faster cycling of Ca^{2+} across the store membrane via the SERCA (sarcoplasmic/endoplasmic-reticulum Ca^{2+}-ATPase) pump, this explanation is frequently referred to as a 'steady-state model'.

There are very convincing sets of data in favour of both explanations. All-or-none emptying of stores has been observed in a series of cell types under a variety of conditions [3,4], and efflux of Ca^{2+} from permeabilized cells has been shown to follow quantal kinetics [5]. Similarly, there are data from imaging of intracellular Ca^{2+}-release events [6] and kinetics of Ca^{2+} release [7] that support a steady-state model. At a mechanistic level, the pure quantal release model demands not only differing sensitivities of stores to $InsP_3$, but also a high level of co-operativity such that a small range of $InsP_3$ concentrations will empty a particular store while leaving others unaffected [2]. Such a difference in sensitivity could arise from structural differences, differences in $InsP_3$-receptor density, intracellular cell environment or, possibly, luminal Ca^{2+} concentration (see below). A mechanism to explain the steady-state model was put forward by one of us (R.F.I. [8]), who proposed that the sensitivity of the $InsP_3$ receptor to $InsP_3$ was controlled by the luminal Ca^{2+} concentration. While this is a feasible idea, and there is some evidence that luminal Ca^{2+} can control the activity of the receptor, there is doubt as to whether or not such controls operate over the right concentration range [9–12]. It seems more likely that apparent control by luminal Ca^{2+} is actually being exerted by released Ca^{2+} near the mouth of the receptor channel or at sites within the channel [13].

Instead of control by luminal Ca^{2+}, a steady-state model therefore seems to demand complex, adaptive kinetic changes in the receptor. Such behaviour could be responsible for the observed complexities in the kinetics of Ca^{2+} release by the $InsP_3$ receptor, and models have been developed that describe the process [14,15]. A very recent model highlights the role of Ca^{2+} in activation and inhibition of Ca^{2+} flux [16]. In general the published models involve effects of Ca^{2+} binding to sites within the channel or to sites in the channel mouth (i.e. on the existence of a Ca^{2+} flux), although there is evidence that the $InsP_3$ receptor can show adaptive behaviour, even under conditions where there is no net movement of Ca^{2+} [17–19].

[1] To whom correspondence should be addressed (e-mail a.dawson@uea.ac.uk).

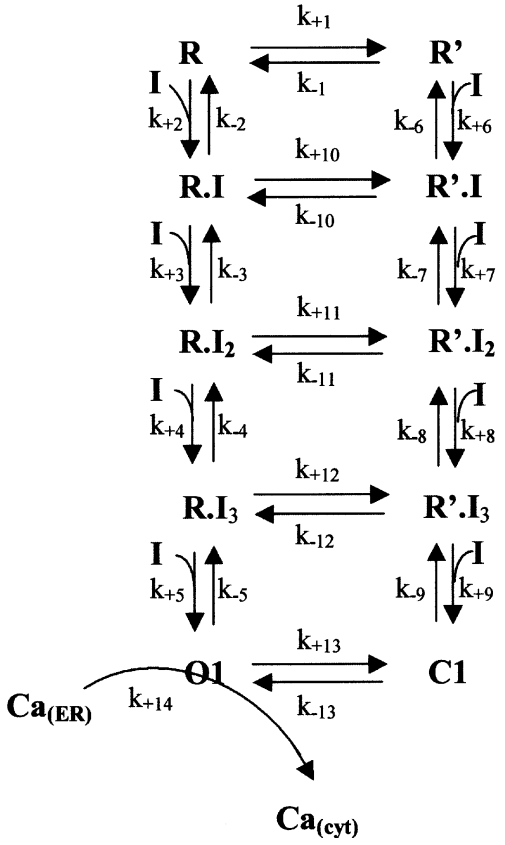
In the case of the structurally homologous ryanodine receptor, adaptive behaviour has been shown at the single-channel level [20], and models have been developed that conform to this [21–23]. It was suggested [22] that similar mechanisms could explain the behaviour of InsP_3 receptors, although this has not been formally examined in relation to the mass of published data on the kinetics of InsP_3-stimulated Ca^{2+} release. We decided to try to devise such a model, starting from the adaptive mechanism suggested for the ryanodine receptor by Sachs et al. [22]. Here we describe how we have built up the model from a simple starting point, where no Ca^{2+} fluxes were involved, to more complex situations that acknowledge the positive and negative feedback from cytosolic Ca^{2+} [24,25] and the inactivation of the receptor in the presence of Ca^{2+} [26]. To our considerable surprise, we finished at a situation where we have a model that shows threshold behaviour with respect to InsP_3 concentration. The threshold value for Ca^{2+} release depends critically on the local concentration of InsP_3 receptors, so that although we set out to construct a steady-state model, we have finished with something that in general not only explains steady-state observations but also conforms to truly quantal release. The model is applicable to both Types 1 and 2 InsP_3 receptors and, with minor modifications to take account of the absence of negative feedback by released Ca^{2+}, to the Type 3 receptor. The model also provides an explanation for how Ca^{2+} fluxes can cease even in the presence of positive feedback from released Ca^{2+}, which appears to be a necessary condition for the production of elementary Ca^{2+} release events such as 'blips' and 'puffs' (the local Ca^{2+} signalling events that do not develop into full, self-propagating waves [26a]).

METHODS

Kinetic simulations were conducted using the kinetic simulator program KSIM, version 2.0, developed by Neil C. Millar. It is currently available at http://wuarchive.wustl.edu/archive2/packages/kinsim/uploads. The archived version will not be permanently available at this web address, but in the event of difficulty, it is available from one of the authors (A.P.D). KSIM simulates the time course of chemical reactions. Reaction models are input as a series of chemical reaction steps (reactive species, their starting concentrations and appropriate rate constants), from which KSIM creates the appropriate differential rate equations. It then solves the rate equations by numerical integration. Outputs from simulations were analysed by EnzFitter (BIOSOFT, Stapleford, Cambridge, U.K.).

RESULTS

Since it was clear that the InsP_3 receptor can show adaptive behaviour under conditions where there is no net Ca^{2+} flux, and therefore positive and negative feedback due to released Ca^{2+} are not possible, we started with a model similar to that suggested by Sachs et al. [22] for the ryanodine receptor. The basic scheme is shown in Scheme 1, which is formally equivalent to that shown in Figure 2 of [22], but with some differences described below. The unliganded receptor is considered to exist in two conformations, R and R′, which are both closed states. R can bind four InsP_3 molecules rapidly, but with low affinity, to form an open state, namely O1. R′ can bind four InsP_3 molecules slowly, but with high affinity, to form a closed state, namely C1. Sachs et al. [22] suggested, in a completely adapting model, that R was in equilibrium with an open, unliganded state, D. However, we know of no evidence to suggest that InsP_3 receptors show channel activity in the absence of InsP_3, so have removed



Scheme 1 An adapting model of the InsP_3 receptor

The Scheme is based on the adaptive model of the ryanodine receptor [22]. The unliganded tetrameric channel can exist in two conformational states, R and R′, in equilibrium. R can bind four molecules of InsP_3 (I) rapidly to form an open state, O1, and R′ can bind four molecules of InsP_3 more slowly, but with higher affinity to form a liganded closed state, C1. The open state, O1, can conduct Ca^{2+} from the inside of the endoplasmic reticulum [$Ca_{(ER)}$] into an external pool [$Ca_{(cyt)}$] with a first-order rate constant k_{+14}. For a more detailed description, see the text. Rate constants are given in Table 1.

the possibility of the high-affinity form being an open state. The consensus view for many years has been that the InsP_3 receptor has to bind up to four InsP_3 molecules to open the channel [27,28], so we built the model around this (although we will see below that co-operative kinetics are possible with only one InsP_3 binding to gate the channel). The successive binding and dissociation rate constants are formulated in the normal way, allowing for the fact that the first InsP_3 to bind has four sites to bind to and one to dissociate from, and so on successively to the fourth InsP_3 to bind, which has one site to bind to and four to dissociate from. The rate constants for binding to the fast, R, form are essentially diffusion-limited. The assumed rate constants are shown in Table 1. They are chosen to give reasonable fits to the sort of kinetic data produced by the rapid superfusion methods of Marchant and Taylor [29], where the feedback effects of released Ca^{2+} are likely to be minimal. However, the model is robust in that it does not depend critically on any absolute values of rate constants, only that the binding of InsP_3 up the left-hand side of the scheme in Scheme 1 is faster and of lower affinity than the binding on the right-hand side. The ratios of the other rate constants are then determined by the need to maintain thermodynamic balance (see legend to Table 1).

Table 1 Values of rate constants applied to the model shown in Scheme 1

The values shown are for the rate constants applied to the kinetic model shown in Scheme 1 to generate the results shown in Figure 1. The numbers down the left-hand side of the Table refer to the subscript numbers of the rate constants in Scheme 1, and the + or − designation refers to the + or − designation in the rate-constant subscripts. k_{+2} is four times faster than k_{+5} because R has four vacant binding sites for InsP_3 to bind to, whereas R.I$_3$ has one. Similarly, O1 has four bound InsP_3 molecules that can dissociate, while R.I has one − hence k_{-5} is four times larger than k_{-2}. The Ca^{2+} efflux rate constant is unidirectional ($k_{-14} = 0$), since it is assumed that the external volume is very high compared with the internal volume, and therefore that $Ca_{(cyt)}$ remains very low compared with $Ca_{(ER)}$. The association rate constants for I binding to R forms are effectively diffusion-limited. Other rate constants are similar to those used in [22], and are chosen to give realistic values for the dose response to InsP_3 and the time course of Ca^{2+} efflux. The ratios of the forward and backward rate constants have to fulfil thermodynamic balance [i.e. $(k_{-1}/k_{+1}) \cdot (k_{+2}/k_{-2}) = (k_{+6}/k_{-6}) \cdot (k_{-10}/k_{+10})$ etc.] for all sets of cyclic reactions.

Rate constant	Value	
	+ (forward)	− (back)
1	1 s^{-1}	100 s^{-1}
2	4000 $\mu M^{-1} \cdot s^{-1}$	1000 s^{-1}
3	3000 $\mu M^{-1} \cdot s^{-1}$	2000 s^{-1}
4	2000 $\mu M^{-1} \cdot s^{-1}$	3000 s^{-1}
5	1000 $\mu M^{-1} \cdot s^{-1}$	4000 s^{-1}
6	400 $\mu M^{-1} \cdot s^{-1}$	10 s^{-1}
7	300 $\mu M^{-1} \cdot s^{-1}$	20 s^{-1}
8	200 $\mu M^{-1} \cdot s^{-1}$	30 s^{-1}
9	100 $\mu M^{-1} \cdot s^{-1}$	40 s^{-1}
10	1 s^{-1}	10 s^{-1}
11	1 s^{-1}	1 s^{-1}
12	10 s^{-1}	1 s^{-1}
13	10 s^{-1}	0.1 s^{-1}
14	100 s^{-1}	0

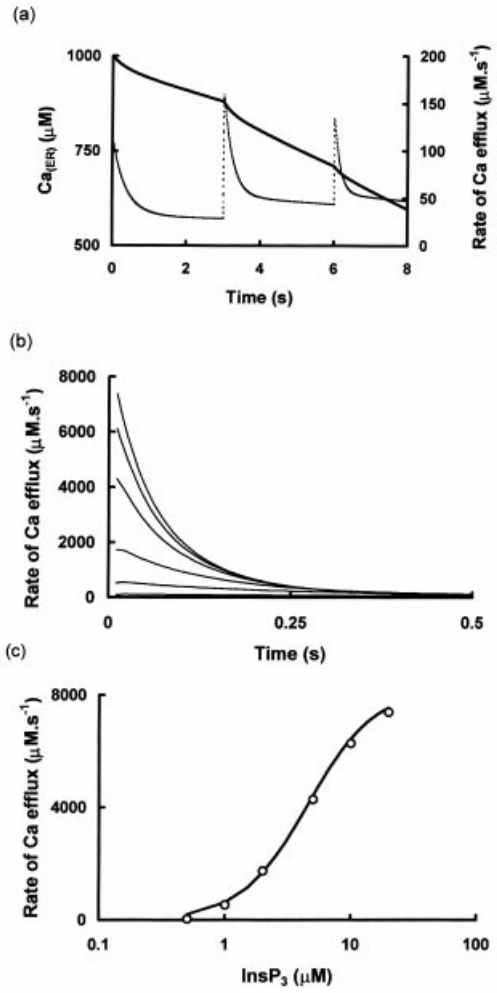
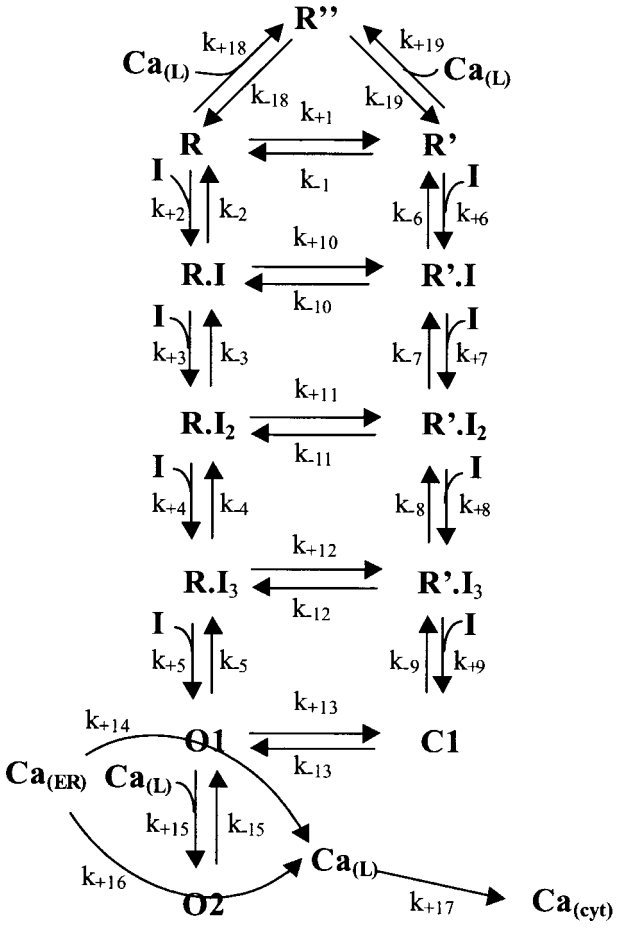


Figure 1 Dependence of Ca^{2+} efflux rate on changes in InsP_3 concentration

(**a**) Shows the response to serial additions of InsP_3 of the model shown in Scheme 1, with the rate constants given in Table 1. At $t = 0$, the concentration of InsP_3 was raised from 0 to 0.5 μM, at $t = 3$ s from 0.5 to 1.0 μM and at $t = 6$ s from 1.0 to 2.0 μM. The heavy line (left-hand axis) shows the change in Ca^{2+} concentration in the store [$Ca_{(ER)}$], whereas the light dotted line (right hand axis) shows the rate of Ca^{2+} efflux. The concentration of InsP_3 receptors was assumed to be 0.01 μM. (**b**) Shows the rate of Ca^{2+} efflux in response to a series of InsP_3 concentrations added at $t = 0$. In ascending order, the InsP_3 additions were (μM): 0.5, 1.0, 2.0, 5.0, 10, 20. (**c**) The dependence of the maximal rate of efflux shown in (**b**) on InsP_3 concentration. The line through the points is fitted to the Hill equation with an h value of 1.7.

The response of the system shown in Scheme 1 to repeated small doses of InsP_3 is shown in Figure 1(a). Successive InsP_3 challenges produce successive increases in the rate of Ca^{2+} efflux, which then relax back to a basal rate. Because the model chosen is not perfectly adapting (for this to happen, R′ has to have the same open probability as O1, while we assume that R′ is closed), the basal rate increases with increasing [InsP_3]. The mechanism of adaptation in the model is eloquently described by Sachs et al. [22], but, put briefly, when an InsP_3 addition is made, the equilibrium between R and O1 is displaced towards O1, causing Ca^{2+} release. The slower equilibria between R and R′, and R′ and C1, then come into play. These pull R into R′ and C1 with a consequent decrease in O1. As well as responding to successive additions of InsP_3, the system also responds transiently to increasing initial doses of InsP_3, in a way similar to that observed by Marchant and Taylor [29]. The behaviour is shown in Figure 1(b). The dose–response curve can be fitted to a Hill equation with a Hill coefficient ('Hill number', h) value of 1.7.

The transient responses generated by this model are consistent with the sort of incremental responses seen by Ferris et al. [17], by Hajnoczky and Thomas [18] and by Renard-Rooney et al. [19], under conditions of no net Ca^{2+} flux, and of Marchant and Taylor [29] under conditions where build-up of Ca^{2+} at the mouth of the channel is minimized. However, as a model for the sort of increment-detection experiments of Meyer and Streyer [2] and of the many other sets of data showing very clear patterns of quantal Ca^{2+} release, the transient responses in Figure 1(a) are not impressive. Accordingly we added on to the model the ability of released Ca^{2+} to feed back positively on its own release. At the same time, we added a dead-end R′–Ca complex (R″) to mimic the inhibition of InsP_3 receptors in the presence of high cytosolic [Ca^{2+}], particularly in the absence of InsP_3 [26]. This, more complex, system is shown in Scheme 2. The main features of the model are: (i) O1 can bind Ca^{2+} to form another open state, O2, with a higher open probability than O1, consistent with the single-channel data of Bezprozvanny et al. [24]; (ii) Ca^{2+} from inside the Ca^{2+} store [$Ca_{(ER)}$] is released into a local domain close to the receptor mouth [$Ca_{(L)}$], from which it diffuses by a simple first-order process into an infinite sink of Ca^{2+} outside [$Ca_{(cyt)}$], which is effectively unchanged during the course of Ca^{2+} efflux; (iii) $Ca_{(L)}$ is the species that binds to the receptor to activate or inhibit it; (iv) both R and R′ can bind $Ca_{(L)}$ to produce an inhibited state, R″. Again, values for the rate constants for Ca^{2+} binding are chosen to be consistent with published data, but the



Scheme 2 Adapting model of the InsP_3 receptor incorporating feedback effects of Ca^{2+} near the channel mouth

The model is an extension of that shown in Scheme 1. It incorporates release of Ca^{2+} into a domain close to the channel mouth [$Ca_{(L)}$], from where it can diffuse into the external medium [$Ca_{(cyt)}$]. The open state, O1, can bind $Ca_{(L)}$ to form another open state, O2, with a tenfold higher open probability (the positive-feedback step). Additionally, R and R′ can bind $Ca_{(L)}$ to form an inactive state, R″, which is not liganded to InsP_3. For the data shown in Figure 3, the following rate constants were used, in addition to those given in Table 1: $k_{+15} = 100\ \mu M^{-1} \cdot s^{-1}$, $k_{-15} = 10\ s^{-1}$, $k_{+16} = 1000\ s^{-1}$, $k_{+17} = 100\ s^{-1}$, $k_{+18} = 1\ \mu M^{-1} \cdot s^{-1}$, $k_{-18} = 0.1\ s^{-1}$, $k_{+19} = 10\ \mu M^{-1} \cdot s^{-1}$, $k_{-19} = 0.01\ s^{-1}$. The flow of $Ca_{(ER)}$ into $Ca_{(L)}$ will be reversible, but in practice, with the rate constants chosen, $Ca_{(L)}$ never rises high enough for the backflow to be significant. The back reactions k_{-14} and k_{-16} have therefore been omitted for clarity.

model withstands wide variations in the absolute values used. Models of Ca^{2+} release with effects due to Ca^{2+} in the channel or at the channel mouth have been explored previously [14–16], but without the adaptive substructure that we have carried forward from Scheme 1. The behaviour of the model shown in Scheme 2 in response to serial challenges with InsP_3 is shown in Figure 2. The response is very strikingly 'quantal' in nature, showing an excellent reproduction of the patterns of Ca^{2+} release described by many workers (see, e.g., [2]). However, what is described is fundamentally a steady-state mechanism. In Figure 2, a low dose of InsP_3 causes partial emptying of an individual store, which retains a slightly higher Ca^{2+} permeability than it had before InsP_3 addition. The reason for the exaggeration of the transient effects shown by the simpler model (Figure 1) is because the conversion of R into O1 causes Ca^{2+} release into the $Ca_{(L)}$ pool. In turn, this drives O1 into O2, increasing $Ca_{(L)}$ even further.

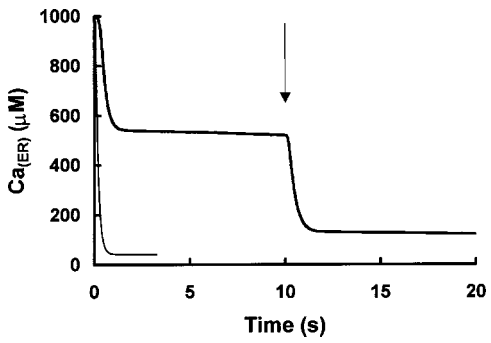


Figure 2 Increment detection by the adaptive model with feedback from released Ca^{2+}

The kinetic model shown in Scheme 2 was used to generate the curves shown. For the thin trace the InsP_3 concentration was raised from 0 to 0.7 μM at $t = 0$. For the heavy trace, InsP_3 was raised from 0 to 0.35 μM at $t = 0$, and from 0.35 to 0.7 μM at the arrow ($t = 10$ s). $Ca_{(ER)}$ was set to 1000 μM, and the concentration of InsP_3 receptors was assumed to be 0.01 μM.

However, ultimately the readjustment of the R ↔ R′ and R′ ↔ C1 equilibria causes O1 to decline as before, resulting in a collapse of $Ca_{(L)}$ and channel closure.

The way the model responded to increments of InsP_3 was sufficiently promising for us to investigate various other aspects of its behaviour. Figure 3 shows the Ca^{2+} release response to a series of InsP_3 concentrations. Particularly at low InsP_3 concentrations, there is a substantial lag phase before the maximal rate of Ca^{2+} release is reached. This is in agreement with many rapid kinetic studies of Ca^{2+} release [27–29]. The dose–response curve for the effect of InsP_3 concentration (Figure 3b) shows extremely co-operative kinetics ($h \approx 7$), because of the way in which released $Ca_{(L)}$ pulls O1 over into O2. The fit to the Hill plot is not good at low [InsP_3], and a rather better description of the release kinetics is that they show threshold behaviour – that is, nothing happens until a threshold [InsP_3] is reached. Such behaviour has been described by Parker et al. [30–32] for Ca^{2+} release from small areas of *Xenopus* oocytes using flash-release of caged InsP_3. The significance of this observation is discussed below.

The model as set up in Scheme 2 has no Ca^{2+}-binding sites on the luminal side of the membrane, and is therefore insensitive directly to changes in $Ca_{(ER)}$. This is in accord with observations under conditions where care is taken to avoid changes in Ca^{2+} near the mouth of the channel [33]. However, there are data suggesting that the $Ca_{(ER)}$ concentration can affect the release of Ca^{2+} by binding to cytosolic sites [11]. We mimicked this behaviour by running the simulation shown in Figure 3(a) at two different $Ca_{(ER)}$ concentrations. The results are plotted in Figure 3(b). In agreement with the observations of Horne and Meyer [13], decreasing the luminal Ca^{2+} concentration caused a rightward shift in the threshold InsP_3 concentration, since the decreased rate of Ca^{2+} efflux resulted in a lower peak level of $Ca_{(L)}$. This rightward shift also means that a repeat challenge, following wash-out, by a given InsP_3 concentration results in a very much smaller Ca^{2+} release than was caused by the first challenge (results not shown). The insensitivity of stores to repeat stimulation with sub-optimal InsP_3 concentrations has been observed experimentally [33a]. It is noteworthy that the effect of $Ca_{(ER)}$ on efflux is mediated in the model via $Ca_{(L)}$. Experimentally, the effects of luminal Ca^{2+} would therefore depend critically on the level of buffering of Ca^{2+} in the $Ca_{(L)}$ domain. Furthermore, since binding of $Ca_{(L)}$ to O1 is saturable, the effect

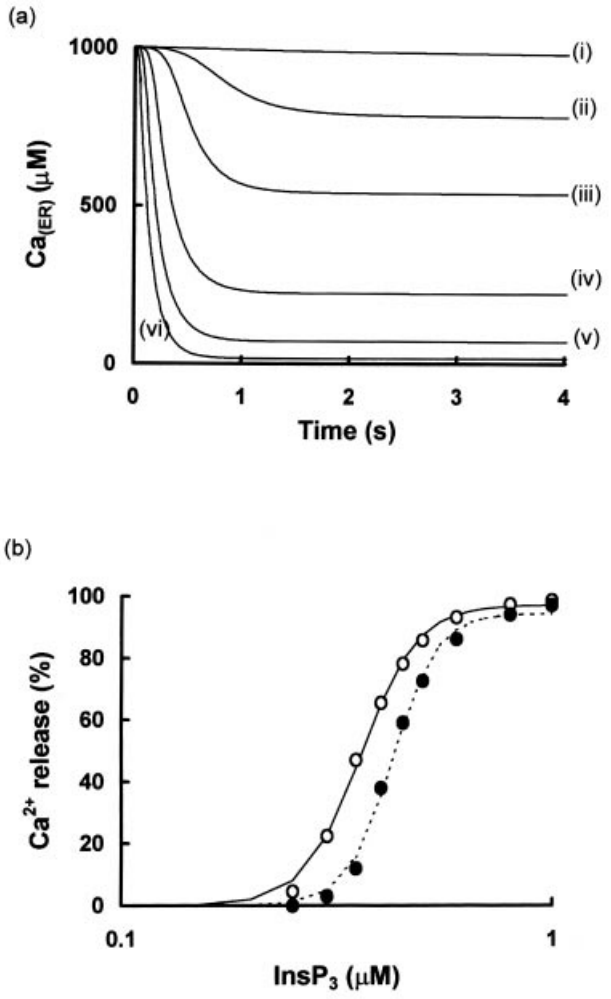


Figure 3 Effects of increasing InsP_3 concentration on Ca^{2+} release

The kinetic model shown in Scheme 2 was used to generate the sets of data shown. The concentration of InsP_3 receptors was assumed to be 0.01 μM. In (**a**), the value for $Ca_{(ER)}$ was set at 1000 μM, and the InsP_3 concentrations added at $t = 0$ were: (i) 0.25 μM; (ii) 0.3 μM; (iii) 0.35 μM; (iv) 0.45 μM; (v) 0.6 μM; (vi) 1 μM. In (**b**) the response is plotted as a percentage of the starting $Ca_{(ER)}$ released after 10 s, by which time Ca release has ceased (see **a**). Starting concentrations of $Ca_{(ER)}$: ○, 1000 μM; ●, 500 μM. The lines fitted through the points are best fits to the Hill equation, with h values of 6.6 (—) and 8.3 (- - -).

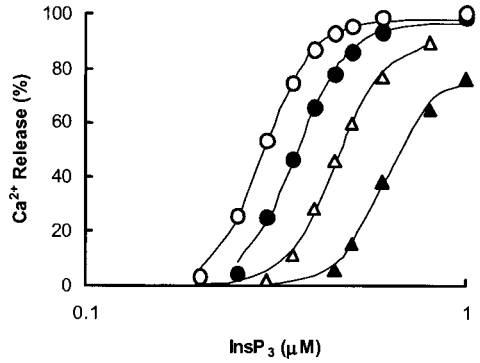


Figure 4 Effect of changing InsP_3-receptor density on Ca^{2+} release

The kinetic model shown in Scheme 2 was used to generate efflux curves at a series of InsP_3 receptor concentrations. In all cases the initial $Ca_{(ER)}$ was 1000 μM. InsP_3 receptor concentrations were (μM): ○, 0.02; ●, 0.01; △, 0.005; ▲, 0.0025. The lines through the points were best fits to the Hill equation, with h values of 6.8 (○), 6.6 (●), 6.8 (△) and 7.2 (▲).

of changing $Ca_{(ER)}$ on efflux decreases as $Ca_{(ER)}$ (and therefore $Ca_{(L)}$) increases (results not shown). The concentration range in which changes in $Ca_{(ER)}$ will cause changes in the EC_{50} for InsP_3 depends (in the model) on the values of the various rate constants chosen. However, experimentally there is some agreement that the effect of $Ca_{(ER)}$ on EC_{50} for InsP_3 is much more pronounced at very low store loadings, as would be predicted from the model [10–12].

Another factor that has been suggested to contribute to the kinetic patterns of Ca^{2+} release is the different densities of InsP_3 receptors on different store types [28,34]. We have modelled the effects of InsP_3-receptor concentrations in the simulations presented in Figure 4. The model uses the effective receptor concentration in three-dimensional space (i.e., expressed in conventional molarity), but in reality this reflects stores with different numbers of receptors per area of membrane, so the numbers chosen are arbitrary. What the simulation shows is that the EC_{50} for InsP_3 depends critically on the relative value chosen for receptor concentration. Lower receptor concentrations cause a rightward shift in the dose response. A very striking aspect of the data is that they now demonstrate true quantal behaviour between different stores. For example, at a concentration of 0.3 μM InsP_3, a store with a receptor density of 0.02 μM will discharge its Ca^{2+} load almost completely, whereas one with a receptor density of 0.005 μM will be essentially unaffected. The model is delivering precisely the requirements that were set out for quantal behaviour in the Introduction – namely extreme co-operativity and stores with differing InsP_3 sensitivities. The underlying cause of the shift in dose response with receptor density is the extent to which adjacent receptors contribute to their collective cloud of $Ca_{(L)}$ – a mechanism that is also believed to be involved in the transition from local to global Ca^{2+} signals [26a]. It is an interesting observation in this context that the reports in which the partial release of Ca^{2+} by InsP_3 shows most clear steady-state characteristics are also those in which the effect of luminal Ca^{2+} on InsP_3 sensitivity is most clearly seen [7,36]. If luminal Ca^{2+} effects are only prominent at low levels of Ca^{2+} pool filling [11], then in these experiments [7,36] an overall low level of

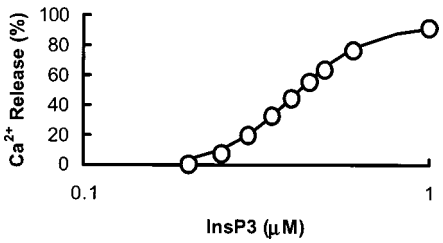


Figure 5 Ca^{2+} release from a non-homogeneous population of Ca^{2+} pools

The curve shown is a composite made from the four dose–response curves shown in Figure 4.The amount of Ca^{2+} released for each receptor density was taken from the data in Figure 4, and the total release then calculated by simple addition of the four component values at each InsP_3 concentration. This was then expressed as a percentage of the total amount of Ca^{2+} in the stores, assuming the same Ca^{2+} concentration and store volume in each case. The curve through the data points is fitted to the Hill equation, with an h value of 4.3.

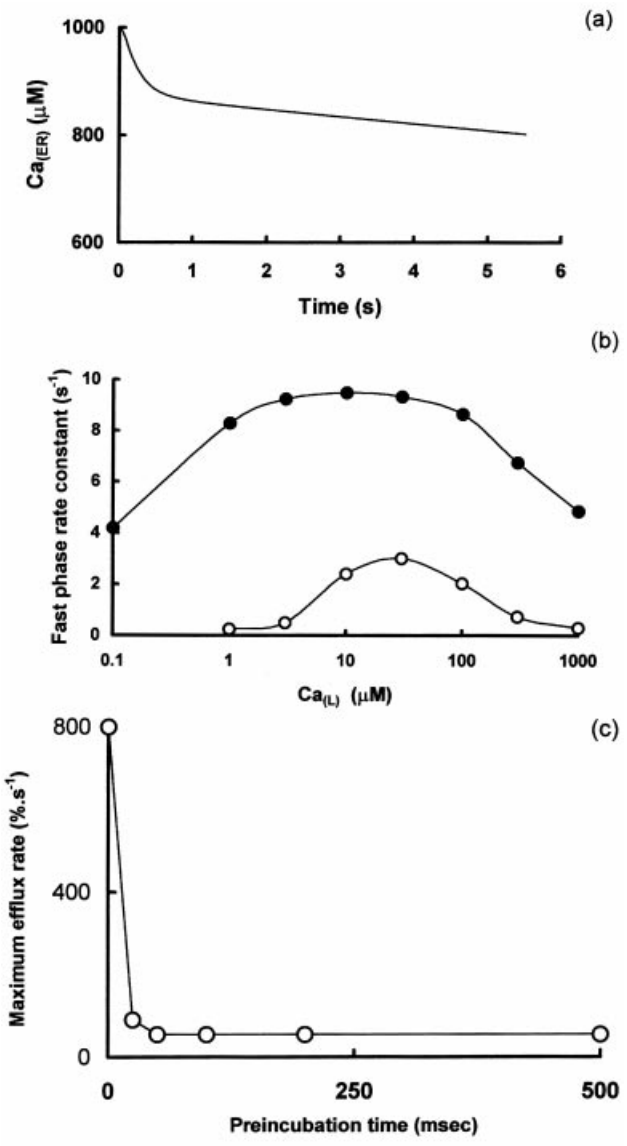


Figure 6 Effects of $Ca_{(L)}$ on Ca^{2+} release

Scheme 2 was modified so that the value of $Ca_{(L)}$ could be set at a pre-determined value, and $Ca_{(ER)}$ could pass directly to $Ca_{(cyt)}$ without contributing to $Ca_{(L)}$. The values of the rate constants were: for O1 converting $Ca_{(ER)}$ into $Ca_{(cyt)}$, $k_{+14} = 100\ s^{-1}$; for O2 converting $Ca_{(ER)}$ into $Ca_{(cyt)}$, $k_{+16} = 1000\ s^{-1}$. Other parameters were exactly as for Scheme 2, except that $k_{+17} = 0$ [$Ca_{(L)}$ cannot diffuse into $Ca_{(cyt)}$]. (**a**) Biphasic Ca^{2+} release when $Ca_{(L)}$ is clamped at 10 μM and the concentrations of $InsP_3$ and $InsP_3$ receptor were 0.2 and 0.01 μM respectively. The generated curve is identical with a curve fitted to two exponentials in EnzFitter, with an initial fast phase of magnitude 138 μM $Ca_{(ER)}$ and rate constant of 2.4 s^{-1} and a slow phase, magnitude 195 μM $Ca_{(ER)}$, rate constant 0.086 s^{-1}. (**b**) Effects of changing $Ca_{(L)}$ on the fast-phase rate constant. $InsP_3$ concentration was either 0.2 μM (○) or 2.0 μM (●). (**c**) Shows the time course of inhibition of the receptor in the presence of $Ca_{(L)}$ and the absence of $InsP_3$. The system was pre-incubated for various lengths of time with 100 μM $Ca_{(L)}$ in the absence of $InsP_3$. Efflux was then started by the addition of 10 μM $InsP_3$.

pool loading may reduce the heterogeneity between pools and the positive feedback of cytosolic Ca^{2+} sufficiently for the steady-state behaviour of individual pools to be exposed. Furthermore, the effect of receptor density decreases as the density increases. This can be seen in Figure 4, where the curves for twofold changes in receptor concentration become closer together towards the left. Once the receptor density is sufficiently high, the collective cloud of $Ca_{(L)}$ saturates the neighbouring O1 species and the release properties become independent of receptor density. It may be that this provides an explanation for the observations of Davis et al. [37] that massive overexpression of $InsP_3$ receptors has only a modest effect on agonist-induced Ca^{2+}-release profiles.

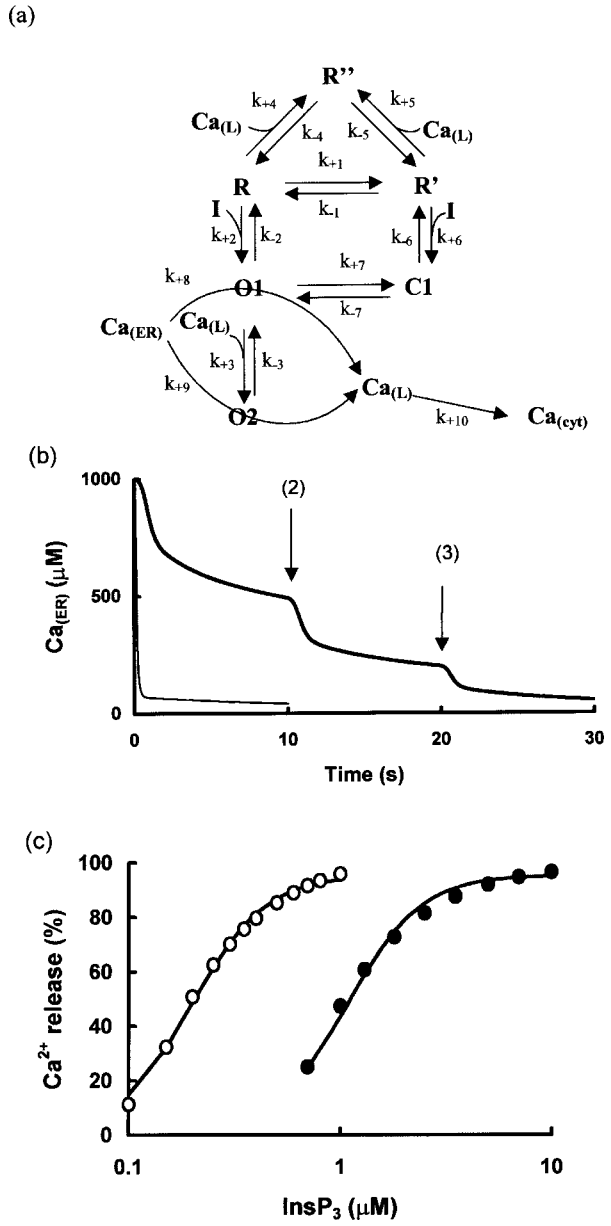
An apparent discrepancy between the simulations generated here and much published experimental data is the very high value for h predicted by the model. However, it should be noted that, in the data published by Parker et al. [30–32], where release is measured in a very small region of a *Xenopus* oocyte, there is extreme co-operativity for the relationship between $InsP_3$ concentration and amount of Ca^{2+} released, and the authors' rationalization of the observation is the same as the one underlying the data here. A threshold $InsP_3$ concentration leads to regenerative feedback. A clear possibility is that, in the majority of measurements of Ca^{2+} release, what is being measured is release from a wide variety of stores, with different receptor densities and Ca^{2+} contents, and the resulting dose–response curve is an average of a range of individual values. We have mimicked this situation by taking the data from a range of receptor densities, summing them and then fitting the data to a Hill plot. The result is shown in Figure 5. The composite data shifts h from about 7 when individual stores are examined to 4 when the average of a number of stores is used. This finding raises concerns about the utility of h in attempts to understand the mechanism of $InsP_3$-dependent Ca^{2+} release, a topic we return to in the Discussion section.

We have also considered the effects of [$Ca_{(L)}$] on the kinetics of Ca^{2+} release. The time course of Ca^{2+} release is rather different when $Ca_{(L)}$ is held constant compared with that seen when $Ca_{(L)}$ changes according to the dynamics of release (as in Figures 2 or 3a). Significantly, the release process becomes biphasic, with an initial fast phase being followed by a much slower phase (Figure 6a). The slow phase is due to the presence of a significant concentration of $Ca_{(L)}$ keeping a finite proportion of the receptors in the O2 form after the initial transient. Experimentally, the biphasic nature of Ca^{2+} release has been frequently reported [2,43–45], where an initial fast phase of Ca^{2+} release is followed by a slower phase with a rate constant about tenfold lower than the initial rate constant. In Figure 6(b), we show the effects of changing [$Ca_{(L)}$] on the rate constant for Ca^{2+} efflux at constant [$InsP_3$]. The data fall on a bell-shaped curve, consistent with published data [24,25]. Also, in agreement with more recent observations, inhibition by high values of $Ca_{(L)}$ is much less marked at higher [$InsP_3$] [41,42]. As in the observations of Adkins and Taylor [26], preincubation with [$Ca_{(L)}$] in the absence of $InsP_3$ results in a rapid inactivation, as R and R′ are converted into R″ (Figure 6c). When $InsP_3$ is present, this transition is much reduced, since during the Ca^{2+} release phase most of the receptor is in the forms O1, O2 and C1 and the intermediates leading to them, resulting in a low concentration of R′.

DISCUSSION

The kinetic model of the $InsP_3$ receptor analysed here demonstrates a series of properties that could provide answers to some of the long-running arguments about the nature of $InsP_3$-dependent Ca^{2+} release. However, it also casts doubt on some long-held assumptions.

From a positive point of view, we have shown that the adaptive model suggested by Sachs et al. [22] to explain the behaviour of the ryanodine receptor channel can also be extended



Scheme 3 Adaptive channel model based on the occupation of one InsP_3 binding site being required to open the channel

The model is similar in pattern to the one shown in Scheme 2, except that only one InsP_3 molecule has to bind to form the open (O1) state. As before, $Ca_{(L)}$ can bind to O1 to form O2, with a higher open probability, and to R and R′ to form the closed (inhibited) state R″. (**a**) General kinetic scheme. The rate constants used in the simulations have the following values (dimensions of first-order rate constants, s^{-1}; second-order rate constants, $\mu M^{-1} \cdot s^{-1}$): $k_{+1} = 1$, $k_{-1} = 10$; $k_{+2} = 100$, $k_{-2} = 12000$; $k_{+3} = 100$, $k_{-3} = 10$; $k_{+4} = 1$, $k_{-4} = 1$; $k_{+5} = 10$, $k_{-5} = 1$; $k_{+6} = 2$, $k_{-6} = 2.4$; $k_{+7} = 10$, $k_{-7} = 1$; $k_{+8} = 500$; $k_{+9} = 5000$; $k_{+10} = 500$. (**b**) Quantal response of the model in (**a**) to repeat challenges of InsP_3. $Ca_{(ER)}$ was 1000 μM, and the receptor concentration was 0.01 μM. Heavy trace: at $t = 0$, 0.2 μM InsP_3 was added. At the arrow marked '(2)' the concentration was raised to 0.4 μM, and at the arrow marked '(3)' from 0.4 to 1.0 μM. Light trace: 1.0 μM InsP_3 added at $t = 0$. (**c**) Dose response to InsP_3 of the model in (**a**) at receptor concentrations of 0.01 μM (○) or 0.002 μM (●). In both cases, the curve through the points is fitted to the Hill equation, with an h value of 2.5.

to explain the kinetics of InsP_3 receptors. The model shows adaptive responses to InsP_3 additions in the absence of net Ca^{2+} flux, and these adaptive responses are greatly magnified by the inclusion of Ca^{2+} movements that lead to local increases in [Ca^{2+}] near the mouth of the channel. Submaximal doses of InsP_3 lead to partial release of Ca^{2+} from a store in a rapid transient. The transient is followed by a state where the basal leakage of Ca^{2+} from the store is, comparatively, extremely low in the ongoing presence of InsP_3. This corresponds to the steady-state model of Ca^{2+} release, which has been supported by various lines of evidence, including the finding of a small, but measurable, increase in Ca^{2+} permeability that follows the rapid release of a fraction of stored Ca^{2+} [6,7]. However, the model also demonstrates very high levels of co-operativity for the dose response to InsP_3, including a threshold behaviour, where there is essentially no Ca^{2+} release seen until a particular level of InsP_3 is reached. Above this level, release increases very rapidly with InsP_3 concentration. We show that the threshold depends on the Ca^{2+} content of the store (if the concentration of Ca^{2+} at the channel mouth is not controlled completely by cytosolic Ca^{2+} buffering), but also, importantly, on the receptor density of a particular store. This behaviour allows us to model situations where a particular concentration of InsP_3 leads to complete emptying of some stores while others are unaffected, as has frequently been observed (see, e.g., [3,4]). In addition, it would also provide a basis for the nature of the subcellular regions that are particularly likely to act as Ca^{2+} blip or puff sites [38]. Any relatively small variations in InsP_3 receptor density would render those regions of the cell more or less excitable.

As well as providing a structural basis for puff sites, the model also provides a mechanism for blip and puff termination. A problem with positive feedback systems is that they are inherently unstable, and it is difficult to see how, once started, Ca^{2+} release does not continue in an autocatalytic manner until the store is empty. Inhibition by high cytosolic [Ca^{2+}] [24,25] serves only to stabilize the level of channel mouth Ca^{2+} at a maximum value, rather than cause it to decrease back to resting levels and stop efflux. The model described here, however, accounts for the cessation of efflux, since the readjustment of the $R \leftrightarrow R'$ and $O1 \leftrightarrow C1$ equilibria lead to diminution of $Ca_{(L)}$, loss of positive feedback and termination of the signal.

Fundamental to the model is the postulate that the receptor can exist in two significantly different conformational states, only one of which can transform to open states. Single-channel data showing multiple open and closed times [39] are consistent with this postulate, as is the recent structural data derived from electron microscopy of the purified receptor which shows two very different conformational states [40].

In a more negative sense, the model raises serious questions about the number of InsP_3 molecules that have to bind to a tetrameric receptor in order to gate the channel. For many years it has been known that, while binding of InsP_3 to its receptor is not co-operative, release of Ca^{2+} does show co-operativity between InsP_3 molecules, with h values in the region of 2 or 3. This was explained by the assumption that, although InsP_3 binding sites did not interact, several (probably four) subunits had to be occupied by InsP_3 before the channel would open. This was, of course, also the assumption on which the model used here was based. The observations derived from analysis of the model, while not contradicting the assumption, lead to doubt about its experimental basis. To illustrate the problem, Scheme 3(a) shows a simplified kinetic scheme, similar to that of Scheme 2, but with only one InsP_3 molecule needed to open the channel. This model behaves very similarly to the more complex scheme in terms of increment detection and effects of receptor density (Schemes 3b and 3c) but, significantly, shows h values of substantially higher than 1 even though only one InsP_3 molecule is required to gate the channel. This kinetic behaviour arises from the co-operativity between InsP_3 and $Ca_{(L)}$ in causing an increased rate of efflux.

Coupled with the suggestion (Figure 5) that h could also be generated as a composite value from heterogeneous stores, we are left with a problem in the interpretation of h values. Single-channel experiments, when Ca^{2+} is not the current carrier and where feedback from transported Ca^{2+} should be nil, should give true h values for the relationship between open probability and $Ins P_3$ concentration. Recent determinations using this method give h values of 4 [41] or 2 [42], although the former is based on $Ins P_3$-dependent release of channel inhibition by cytosolic Ca^{2+}.

There are features of the kinetics of Ca^{2+} release that we have not directly addressed in the studies reported above. Although the kinetic scheme shown in Scheme 2 appears complex, the reality could be even worse. We have deliberately chosen to keep the model as simple as is necessary to reveal its properties. Therefore we have not considered the possibility of any of the complexes with less than four bound $Ins P_3$ molecules forming open states, or interacting with $Ca_{(L)}$ to do so. Nor have we considered the circumstance where up to four $Ca_{(L)}$ ions have to bind to O1 to convert it into O2, as suggested by the single-channel data published by Mak et al. [41]. The latter would be expected to generate even steeper dependence of efflux rate on $Ins P_3$ concentration than we have demonstrated here. Furthermore, we have not yet considered in detail the different kinetic properties shown by the different isoforms of the $Ins P_3$ receptor. Swatton and Taylor [46], measuring rapid kinetics of Ca release, have recently shown very different Ca^{2+} inhibition patterns in the Type 2 and Type 3 receptors: $Ins P_3$ protects against Ca^{2+} inhibition in Type 2 and not in Type 3 receptors. While these differences may represent major differences in mechanism, it may be that they arise from rather less fundamental differences in rate constants in a kinetic scheme such as the one we describe here. It would be expected, for example, that the existence of a kinetically significant direct route from the species O2 to R″ would cause major changes in the kinetics of Ca^{2+} inhibition.

In the cell, it is very likely that the $Ins P_3$ receptor concentration could be comparable with the $Ins P_3$ concentration, particularly in areas of the cell with high local receptor density. In the present paper, we have deliberately side-stepped this issue by choosing receptor concentrations that are low compared with the applied $Ins P_3$ concentration. However, such a situation would further emphasize the threshold behaviour of the model described, while adding substantially to the complications of interpreting the behaviour of the model.

The principle behind the model for the $Ins P_3$ receptor described here is that of a receptor responding to changes in concentration of its interacting ligand, rather than to absolute concentration. This could be an important feature of signalling systems in general. Indeed, the adapting model of the ryanodine receptor on which our current model is based [22] was itself derived from modelling of bacterial chemotactic receptors, systems that allow organisms to respond to concentration gradients [47].

We thank Neil C. Millar for providing the program KSIM as freeware (ksim1.zip). We also thank Dr Richard Tregear for his help and extreme tolerance during our early attempts to model the $Ins P_3$ receptor, and Dr Sheila Dargan and Professor Colin Taylor for helpful advice and discussions. R.F.I. is supported by The Royal Society. A.P.D. and E.J.A.L. thank the Wellcome Trust for financial support.

REFERENCES

1 Muallem, S., Pandol, S. J. and Beeker, T. G. (1989) Hormone-evoked calcium release from intracellular stores is a quantal process. J. Biol. Chem. **264**, 205–212
2 Meyer, T. and Stryer, L. (1991) Transient calcium release induced by successive increments of inositol 1,4,5-trisphosphate. Proc. Natl. Acad. Sci. U.S.A. **87**, 3841–3845
3 Bootman, M. D., Berridge, M. J. and Taylor, C. W. (1992) All-or-nothing Ca^{2+} mobilization from the intracellular stores of single histamine-stimulated HeLa-cells. J. Physiol. (London) **450**, 163–178
4 Shin, D. M., Luo, X., Wilkie, T. M., Miller, L. J., Peck, A. B., Humphreys-Beher, M. G. and Muallem, S. (2001) Polarized expression of G protein-coupled receptors and an all-or-none discharge of Ca^{2+} pools at initiation sites of [Ca^{2+}] waves in polarized exocrine cells. J. Biol. Chem. **276**, 44146–44156
5 Oldershaw, K. A., Nunn, D. L. and Taylor, C. W. (1991) Quantal Ca^{2+} mobilization stimulated by inositol 1,4,5-trisphosphate in permeabilized hepatocytes. Biochem. J. **278**, 705–708
6 Loomis-Husselbee, J. W. and Dawson, A. P. (1993) A steady-state mechanism can account for the properties of inositol 2,4,5-trisphosphate-stimulated Ca^{2+} release from permeabilized L1210 cells. Biochem. J. **289**, 861–866.
7 Tanimura, A. and Turner, R. J. (1996) Calcium release in HSY cells conforms to a steady-state mechanism involving regulation of the inositol 1,4,5-trisphosphate receptor Ca^{2+} channel by luminal [Ca^{2+}]. J. Cell. Biol. **132**, 607–616
8 Irvine, R. F. (1991) "Quantal" Ca^{2+} release and the control of Ca^{2+} entry by inositol phosphates: a possible mechanism. FEBS Lett. **263**, 5–9
9 Combettes, L., Claret, M. and Champeil, P. (1992) Do submaximal $Ins P_3$ concentrations only induce the partial discharge of permeabilised hepatocyte calcium pools because of the concomitant reduction in intraluminal Ca^{2+} concentration? FEBS Lett. **301**, 287–290
10 Parys, J. B., Missiaen, L., De Smedt, H. and Casteels, R. (1993) Loading dependence of inositol 1,4,5-trisphosphate-induced Ca^{2+} release in the clonal cell line A7r5. J. Biol. Chem. **268**, 25205–25212
11 Combettes, L., Cheek, T. R. and Taylor, C. W. (1996) Regulation of inositol trisphosphate receptors by luminal Ca^{2+} contributes to quantal Ca^{2+} mobilization. EMBO J. **15**, 2086–2093
12 Beecroft, M. D. and Taylor, C. W. (1997) Incremental Ca^{2+} mobilization by inositol trisphosphate receptors is unlikely to be mediated by their desensitization or regulation by luminal or cytosolic Ca^{2+}. Biochem. J. **326**, 215–220
13 Horne, J. H. and Meyer, T. (1995) Luminal Ca^{2+} regulates the inositol trisphosphate receptor of rat basophilic leukaemia cells at a cytosolic site. Biochemistry **34**, 12738–12746
14 Swillens, S., Combettes, L. and Champeil, P. (1994) Transient inositol 1,4,5-trisphosphate-induced Ca^{2+} release: a model based on regulatory Ca^{2+} binding sites along the permeation pathway. Proc. Natl. Acad. Sci. U.S.A. **91**, 10074–10078
15 Dupont, G. and Swillens, S. (1996) Quantal release, incremental detection, and long-period Ca^{2+} oscillations in a model based on regulatory Ca^{2+} binding sites along the permeation pathway. Biophys. J. **71**, 1714–1722
16 Sneyd, J. and Dufour, J.-F. (2002) A dynamic model of the Type-2 inositol trisphosphate receptor. Proc. Natl. Acad. Sci. U.S.A. **99**, 2398–2403
17 Ferris, C. D., Cameron, A. M., Huganir, R. L. and Snyder, S. (1992) Quantal Ca^{2+} release by purified reconstituted inositol 1,4,5-trisphosphate receptors. Nature (London) **356**, 350–352
18 Hajnoczky, G. and Thomas, A. P. (1994) The inositol trisphosphate calcium channel is inactivated by inositol trisphosphate. Nature (London) **370**, 474–477
19 Renard-Rooney, D. C., Hajnoczky, G., Seitz, M. B., Schneider, T. G. and Thomas, A. P. (1993) Imaging of inositol 1,4,5-trisphosphate-induced Ca^{2+} fluxes in single permeabilized hepatocytes – demonstration of both quantal and nonquantal patterns of Ca^{2+} release. J. Biol. Chem. **268**, 23601–23610
20 Gyorke, S. and Fill, M. (1993) Ryanodine receptor adaptation – control mechanism of Ca^{2+}-induced Ca^{2+} release in heart. Science **260**, 807–809
21 Cheng, H., Fill, M., Valdivia, H. and Lederer, W. J. (1995) Models of Ca^{2+} release channel adaptation. Science **267**, 2009–2010
22 Sachs, F., Qin, F. and Palade, P. (1995) Models of Ca^{2+} release channel adaptation. Science **267**, 2010–2011
23 Zahradnikova, A. and Zahradnik, I. (1996) A minimal gating model for the cardiac calcium release channel. Biophys. J. **71**, 2996–3012
24 Bezprozvanny, I., Watras, J. and Ehrlich, B. E. (1991) Bell-shaped calcium-response curves of Ins(1,4,5)P_3-gated and calcium-gated channels from endoplasmic-reticulum of cerebellum. Nature (London) **351**, 751–754
25 Finch, E. A., Turner, T. J. and Goldin, S. M. (1991) Calcium as a coagonist of inositol 1,4,5-trisphosphate induced calcium release. Science **252**, 443–446
26 Adkins, C. E. and Taylor, C.W (1999) Lateral inhibition of inositol 1,4,5-trisphosphate receptors by cytosolic Ca^{2+}. Curr. Biol. **9**, 1115–1118
26a Bootman, M. D., Niggli, E., Berridge, M. J. and Lipp, P. (1997) Imaging the hierarchical Ca^{2+} signalling system in HeLa cells. J. Physiol. (London) **499**, 307–314
27 Meyer, T., Wensel, T. and Stryer, L. (1990) Kinetics of calcium channel opening by inositol 1,4,5-trisphosphate. Biochemistry **29**, 32–37
28 Marchant, J. S. and Taylor, C. W. (1998) Rapid activation and partial inactivation of inositol trisphosphate receptors by inositol trisphosphate. Biochemistry **37**, 11524–11533

29 Marchant, J. S. and Taylor, C. W. (1997) Cooperative activation of IP_3 receptors by sequential binding of IP_3 and Ca^{2+} safeguards against spontaneous activity. Curr. Biol. **7**, 510–518

30 Parker, I. and Ivorra, I (1990) Localized all-or-none calcium liberation by inositol trisphosphate. Science **250**, 977–979

31 Parker, I. and Ivorra, I (1993) Confocal microfluorimetry of Ca^{2+} signals evoked in *Xenopus* oocytes by photoreleased inositol trisphosphate. J. Physiol. (London) **461**, 133–165

32 Parker, I., Yao, Y. and Ilyin, V. (1996) Fast kinetics of calcium liberation induced in *Xenopus* oocytes by photoreleased inositol trisphosphate. Biophys. J. **70**, 222–237

33 Bezprozvanny, I and Ehrlich, B. E. (1994) Inositol (1,4,5)-trisphosphate (InsP_3)-gated Ca channels from cerebellum: conduction properties for divalent cations and regulation by intraluminal calcium. J. Gen. Physiol. **104**, 821–856

33a Bootman, M. D., Cheek, T. R., Moreton, R. B., Bennett, D. L. and Berridge, M. J. (1994) Smoothly graded Ca^{2+} release from inositol 1,4,5-trisphosphate-sensitive Ca^{2+} stores. J. Biol. Chem. **269**, 24783–24791

34 Hirose, K. and Iino, M. (1994) Heterogeneity of channel density in inositol-1,4,5-trisphosphate-sensitive Ca^{2+} stores. Nature (London) **372**, 791–794

35 Reference deleted

36 Missiaen, L., DeSmedt, H., Droogmans, G. and Casteels, R. (1992) Ca^{2+} release induced by inositol 1,4,5-trisphosphate is a steady-state phenomenon controlled by luminal Ca^{2+} in permeabilized cells. Nature (London) **357**, 599–602

37 Davis, R. J., Challis, R. A. J. and Nahorski, S. R. (1999) Enhanced purinoceptor-mediated Ca^{2+} signalling in L-fibroblasts overexpressing type 1 inositol 1,4,5-trisphosphate receptors. Biochem. J. **341**, 813–820

38 Thomas, D., Lipp, P., Tovey, S. C., Berridge, M. J., Li, W. H., Tsien, R. Y. and Bootman, M. D. (2000) Microscopic properties of elementary Ca^{2+} release sites in nonexcitable cells. Curr. Biol. **10**, 8–15

39 Thrower, E. C., Mobasheri, H., Dargan, S., Marius, P., Lea, E. J. A. and Dawson, A. P. (2000) Interaction of luminal calcium and cytosolic ATP in the control of type 1 inositol (1,4,5)-trisphosphate receptor channels. J. Biol. Chem. **275**, 36049–36055

40 Hamada, K., Miyata, T., Mayanagi, K., Hirota, J. and Mikoshiba, K. (2002) Two-state conformational changes in inositol 1,4,5-trisphosphate receptor regulated by calcium. J. Biol. Chem. **277**, 21115–21118

41 Mak, D. D., McBride, S. and Foskett, J. K. (1998) Inositol 1,4,5-trisphosphate activation of inositol trisphosphate receptor Ca^{2+} channel by ligand tuning of Ca^{2+} inhibition. Proc. Natl. Acad. Sci. U.S.A. **95**, 15821–15825

42 Moraru, I. I., Kaftan, E. J., Ehrlich, B. E. 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

43 Hirota, J., Michikawa, T., Miyawaki, A., Furuichi, T., Okura, I. and Mikoshiba, K. (1995) Kinetics of calcium-release by immunoaffinity-purified inositol 1,4,5-trisphosphate receptor in reconstituted lipid vesicles. J. Biol. Chem. **270**, 19046–19051

44 Mezna, M. and Michelangeli, F. (1996) The effects of inositol 1,4,5-trisphosphate (InsP_3) analogues on the transient kinetics of Ca^{2+} release from cerebellar microsomes. J. Biol. Chem. **271**, 31818–31823

45 Loomis-Husselbee, J. W., Cullen, P. J., Dreikhausen, U. E., Irvine, R. F. and Dawson, A. P. (1996) Synergistic effects of inositol 1,3,4,5-tetrakisphosphate on inositol 2,4,5-trisphosphate-stimulated Ca^{2+} release do not involve direct interaction of inositol 1,3,4,5-tetrakisphosphate with inositol trisphosphate-binding sites. Biochem. J. **314**, 811–816

46 Swatton, J. E. and Taylor, C. W. (2002) Fast biphasic regulation of Type 3 inositol trisphosphate receptors by cytosolic calcium. J. Biol. Chem. **277**, 17571–17579

47 Knox, B. E., Devreotes, P. N., Goldbeter, A. and Segel, L. A. (1986) A molecular mechanism for sensory adaptation based on ligand-induced receptor modification. Proc. Natl. Acad. Sci. U.S.A. **83**, 2345–2349

Received 14 August 2002/4 December 2002; accepted 13 December 2002
Published as BJ Immediate Publication 13 December 2002, DOI 10.1042/BJ20021289