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Review Hypothesis

'Quantal' Ca^{2+} release and the control of Ca^{2+} entry by inositol phosphates – a possible mechanism

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The release of Ca^{2+} from intracellular stores by sub-optimal doses of inositol trisphosphate has been shown to be dose-related ('quantal'), and a simple model is proposed here to account for this phenomenon. It is suggested that there is a regulatory Ca^{2+}-binding site on, or associated with, the luminal domain of the $InsP_3$ receptor, which allosterically controls Ca^{2+} efflux, and the affinity for Ca^{2+} of that site is modulated by $InsP_3$ binding to the cytoplasmic domain of the receptor; a similar mechanism applied to the ryanodine receptor might also explain some aspects of Ca^{2+}-induced Ca^{2+} release. The stimulated entry of Ca^{2+} into a cell which occurs upon activation of inositide-linked receptors has been variously and confusingly proposed to be regulated by $InsP_3$, $InsP_4$, and/or a 'capacitative' Ca^{2+} pool; the mechanism of $InsP_3$ receptor action suggested here is shown to lead to a potential reconciliation of all these conflicting proposals.

1. 'QUANTAL' Ca^{2+} RELEASE

The release of calcium from inositol(1,4,5)trisphosphate($InsP_3$)-sensitive stores is believed to occur by the binding of $InsP_3$ to a protein which is both a receptor for the inositol phosphate, and a channel capable of letting calcium escape from the intraluminal space to the cytoplasm [1–3]. However, recent evidence [4,5] has revealed an unexpected complexity in the way in which this occurs in permeabilized cells. A sub-optimal concentration of $InsP_3$ might be expected to cause a submaximal opening of the Ca^{2+} channels, with the result being a slower efflux of Ca^{2+}, but leading ultimately to the same net release. Such expected behaviour is indeed seen with sub-optimal concentrations of ionomycin [4,5]. But with $InsP_3$ or its non-hydrolysable analogue $InsP(S)_3$, there is a rapid release of a fraction of the releasable Ca^{2+}, and then only a small amount of Ca^{2+} is liberated further unless more $InsP_3$ is added [4,5]. This phenomenon (termed 'quantal' release by Muallam et al. [4]) certainly makes physiological sense – in this context a rapid attainment of a partially altered steady-state may be preferable to a variable speed of approach to a common end-point – but the mechanism remains obscure.

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It is unlikely that this apparently biphasic release of Ca^{2+} is caused by some form of desensitisation of the $InsP_3$ receptor, as if $InsP_3$ is removed from the receptor due to hydrolysis by $InsP_3$-5-phosphatase (i.e. if it is not protected from hydrolysis by that enzyme), a reuptake of Ca^{2+} occurs [4]. The absence of this reuptake if $InsP_3$ is allowed to remain [4] shows that the receptor (or the channel associated with it) has not desensitized. Yet, the experiments of Taylor and Potter [5] performed in the absence of Ca^{2+}-pumping, show that Ca^{2+} efflux decreases markedly once a certain submaximal amount of Ca^{2+} has been released. A simple explanation for these paradoxes lies in the possibility that the intra-luminal Ca^{2+} concentration may play a major role in controlling the release of Ca^{2+}.

The $InsP_3$ receptor is a transmembrane protein (Fig. 1A) with an $InsP_3$ binding site on the cytoplasmic side [2,6,7]. The central proposal of the hypothesis outlined here is that it also has a Ca^{2+} binding site on its intraluminal side (or there is such a binding site on a protein associated with the $InsP_3$ receptor), with an affinity for Ca^{2+} in the millimolar range (dissociation constant K_1, see Fig. 1B). It is also suggested that the binding of Ca^{2+} to this site increases the affinity of the receptor for $InsP_3$ (i.e. $K_2 < K_3$), and consequently, binding of $InsP_3$ to the receptor increases the affinity of the intraluminal Ca^{2+} binding site ($K_4 < K_1$). Note that the possible co-operative nature of the $InsP_3$ binding [8] has not been included here as it does not qualitatively effect the overall arguments. Once Ca^{2+} is bound to

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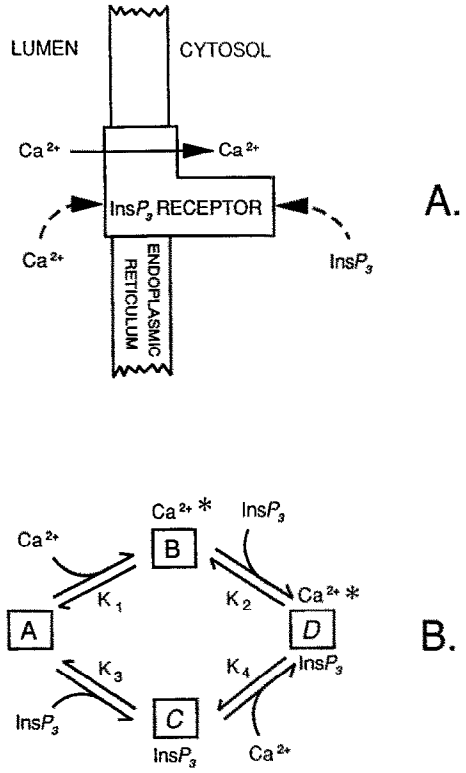

Fig. 1. Proposed mechanism of how the $InsP_3$-receptor modulates Ca^{2+} release. (A). This is a schematic drawing of the $InsP_3$-receptor, emphasising the suggestion put forward here, that there are two allosteric sites on the receptor; one, facing the cytoplasm, binds Ins(1,4,5)P_3, and the other, facing the lumen, binds Ca^{2+}. Below (B) is a proposed modulation of the Ca^{2+} translocation of this receptor by these two ligands. The native receptor is represented as (A), and the forms with an asterisk (B and D) are in a conformation such that the Ca^{2+} channel in the receptor (possibly 4 molecules grouped to form a channel; see [2]) is open and can carry Ca^{2+}.

the site, the Ca^{2+} channel is open, and Ca^{2+} can flood out through the channel. This will manifest itself as a 'leak' preventing the Ca^{2+} pump from raising the free intraluminal Ca^{2+} above a level dictated by the affinity of that site. Strictly speaking, other leaks, including slow Ca^{2+} transport through the 'closed' form of the $InsP_3$ receptor, could prevent intraluminal Ca^{2+} ever getting to this point, but the advantage of this regulatory site controlling the 'set point' is to make the system responsive to low concentrations of $InsP_3$. Alternatively, if only the D-form of the receptor (and not the B-form) permits Ca^{2+} efflux, then this could increase still further the initial sensitivity of the system to $InsP_3$.

Once a steady-state has been established in the absence of $InsP_3$, the subsequent addition of $InsP_3$ will result in the increased formation of a Ca^{2+}-translocating conformation of the receptor (by the generation of some of the form D) and this will lead to a net Ca^{2+} efflux. This efflux will only be transient, however. As the $[Ca^{2+}]$ in the lumen falls, so will the apparent affinity of the $InsP_3$ binding site, and thus the amount of D will decrease until eventually a new set point in intra-luminal Ca^{2+} will be established. The further addition of more $InsP_3$ will once again, at the existing $[Ca^{2+}]$ inside the lumen, drive the formation of more of the 'active' (D) receptor and thus more Ca^{2+} efflux occurs. Thus, one can suggest that a co-operative mechanism of this sort should lead to what would appear as a 'quantal' release of Ca^{2+}.

It is relevant to note in such a context, that given the similarities between the $InsP_3$-receptor and the ryanodine receptor which is responsible in skeletal muscle for Ca^{2+} release [2,6,7,9], a similar principle might be involved in the latter's mode of action. A mechanism similar to that proposed here could perhaps contribute to the transient (= quantal?) nature of release [10], a phenomenon that is lost in reconstitution assays (e.g. [10]). In such reconstitution assays, either Na^+ is the transmitted ion, or 'intraluminal' Ca^{2+} is set at several millimolar from which it will not fall significantly. Ca^{2+}-induced Ca^{2+} release in muscle and other tissues could be explained by a high (micromolar) affinity Ca^{2+}-binding site on the cytoplasmic surface of the receptor, and this, acting co-operatively with the lower affinity intraluminal site, might be expected to cause an autocatalytic, but transient, release of Ca^{2+}. (An effect of caffeine on the intraluminal Ca^{2+}-binding site may explain some of the effects of this drug on Ca^{2+}-induced Ca^{2+} release.)

This model makes some experimentally testable predictions about $InsP_3$-induced Ca^{2+} release. For example, at maximal Ca^{2+} loading, $InsP_3$-sensitive pools will, in response to a sub-optimal dose of $InsP_3$, release from Ca^{2+} to give a lower intraluminal Ca^{2+} level. The model predicts that if the intraluminal loading starts at this lower level, then the same dose of $InsP_3$ will have no effect on Ca^{2+} efflux. Conversely, if the Ca^{2+}-buffering of the pool is increased (for example, by loading with a Ca^{2+} chelator) the total loading can now reach much higher levels, and so the release of Ca^{2+} by a small dose of $InsP_3$ would become much larger and continue until almost all the Ca^{2+} has drained out (because it is the intraluminal *free* Ca^{2+} not the total Ca^{2+} which controls release – the natural Ca^{2+} buffering caused by Ca^{2+}-binding proteins will therefore also influence the quantitative interrelationship between $InsP_3$ and Ca^{2+} release).

2. Ca^{2+} ENTRY

Ca^{2+} entry into the cell, when it is controlled by inositol phosphates rather than by voltage- or receptor-operated channels, is a complex process at present poorly understood (see [12,13] for review), but central to it is the concept that at least part of the regulation of this process involves an intracellular Ca^{2+} pool, and that the concentration of Ca^{2+} inside this pool can influence Ca^{2+} entry. Specifically, when the Ca^{2+} in the

intracellular pool is low, Ca^{2+} entry is increased (see [14] for the original proposal, and [15,16] for more recent evidence supportive of this idea). It is unlikely that the Ca^{2+} content of intracellular pools is the only controlling factor on Ca^{2+} entry, as is clearly shown, for example, by the experiments in [17,18], but it is nevertheless an important influence [14,16,19]. Perhaps the simplest way in which luminal Ca^{2+} could influence Ca^{2+} influx through the plasma membrane would be via a protein which has an intraluminal allosteric Ca^{2+}-binding site, and which interacts with the plasma membrane. This protein could then 'communicate' the intra-luminal Ca^{2+} concentration to the plasma membrane by its interaction with it. As outlined above, the key aspect of the proposed mechanism for 'quantal' Ca^{2+} release in Fig. 1 is to suggest that just such a Ca^{2+}-binding site exists on the $InsP_3$ receptor. Thus it is a plausible extension of that hypothesis to suggest that the $InsP_3$ receptor interacts with a protein in the plasma membrane which controls Ca^{2+} entry (Fig. 2). There is a direct precedent for this in the interrelationship between the ryanodine receptor and the plasma membrane in skeletal muscle. There, the currently popular picture is, that the large cytoplasmic part of the ryanodine receptor spans the space between the sarcoplasmic reticulum and the sarcolemma to provide a close and rapid communication between the two membranes (e.g. [9]). As the $InsP_3$ receptor has a similarly large cytoplasmic component [2] it could do the same thing (Fig. 2).

There are a number of ways in which this could be modelled, but the simplest is to propose that the affinity of the $InsP_3$ receptor for the interaction site with the plasma membrane is modulated by both the Ca^{2+} inside the e.r. lumen and by $InsP_3$, such that low Ca^{2+} in the lumen and high $InsP_3$ promote dissociation of the two proteins. More specifically in the context of Fig. 1B, the B or D forms of the $InsP_3$ receptor would interact with a plasma membrane protein more strongly than the A and C forms, and B would bind more strongly than D. When the two proteins are associated, both the Ca^{2+} entry mechanism through the plasma membrane, and also the Ca^{2+} release through the $InsP_3$ receptor, are blocked, i.e. dissociation of the two proteins promotes Ca^{2+} entry and mobilisation. The result of such interactions would be that $InsP_3$ would increase Ca^{2+} entry (e.g. [20,21]), but to a varying degree, depending on the intraluminal Ca^{2+} content of the $InsP_3$-sensitive pool. At very low intraluminal concentrations of Ca^{2+}, it could be that at least some Ca^{2+} entry could occur in without any $InsP_3$, as has been demonstrated in various tissues (e.g. [16,19]).

The idea that both $InsP_3$ and intraluminal Ca^{2+} influences Ca^{2+} entry in this way becomes particularly attractive if we also consider the role of inositol(1,3,4,5)tetrakisphosphate ($InsP_4$). Most of the experimental data concerning this inositol phosphate can be accounted for in this context by simply suggesting that the protein in the plasma membrane with which the $InsP_3$ receptor interacts is the $InsP_4$ receptor (Fig. 2), and that when $InsP_4$ binds to its receptor, it induces in it a conformation that does not favour interaction with the $InsP_3$ receptor (i.e. $InsP_4$ also promotes dissociation of the two proteins). Experimentally, $InsP_4$ can synergise with $InsP_3$ to control intracellular Ca^{2+} (e.g. [22–24]). The clearest set of data, from the internally-perfused mouse lacrimal cell [23,25], show that $InsP_4$ can be absolutely essential for $InsP_3$-induced Ca^{2+} entry, and that in this function it is not interacting with $InsP_3$/$InsP_4$ phosphatase, but probably with the putative $InsP_4$ receptor described in several tissues [26–28]. Furthermore, in the lacrimal cells, there are some intracellular pools which $InsP_3$ can only mobilize if $InsP_4$ is present [25], and this is predicted from the model depicted in Fig. 2 in that these would be the set of Ca^{2+} pools next to the plasma membrane, interacting with that membrane via the $InsP_3$ and $InsP_4$ receptors. (Note that, strictly speaking, the $InsP_4$ receptor could be in the e.r. and modulate the interaction with another protein in the plasma membrane, but the scheme in Fig. 2 is one protein simpler.)

This mechanism therefore now predicts that raised concentrations of $InsP_3$ and $InsP_4$ and lowered intraluminal Ca^{2+} will all contribute to the degree to which the two receptors dissociate, and therefore to which the Ca^{2+} entry mechanism is in an 'active' (Ca^{2+}-carrying) conformation. For example, at full intraluminal Ca^{2+} pool loading, neither $InsP_3$ nor $InsP_4$ alone can induce sufficient dissociation of the two receptors to cause significant Ca^{2+} entry, but together they can; thus if they are both added there is (i) Ca^{2+} entry [23], and (ii) some extra mobilization of Ca^{2+} [29]. However, once the Ca^{2+} pool linked to the plasma membrane has been emptied, it may be that, provided $InsP_3$ is there to keep it empty, $InsP_4$ is no longer required to keep the Ca^{2+} entry mechanism at least partly active, i.e. the low intraluminal Ca^{2+} plus high $InsP_3$ can together result in sufficient dissociation of the two receptors for a significant sustained Ca^{2+} influx. This phenomenon has been shown directly in perfused cells by the demonstration that a 'memory' that $InsP_4$ has previously been present persists for several minutes after the $InsP_4$ is removed [29]. A similar 'memory' may also explain why in other experiments where the tissue is chronically exposed to $InsP_3$ (e.g. [20,30]), a transient $InsP_4$ production at the beginning of the experiment would be sufficient to activate the Ca^{2+} entry mechanism so that, given continuous exposure to $InsP_3$, it is subsequently $InsP_4$-independent.

Other examples of apparently contradictory results from different experimental systems may also be accounted for by this model. For example, there will be, to varying degrees in different tissues, $InsP_3$-sensitive pools of Ca^{2+} not associated with the plasma mem-

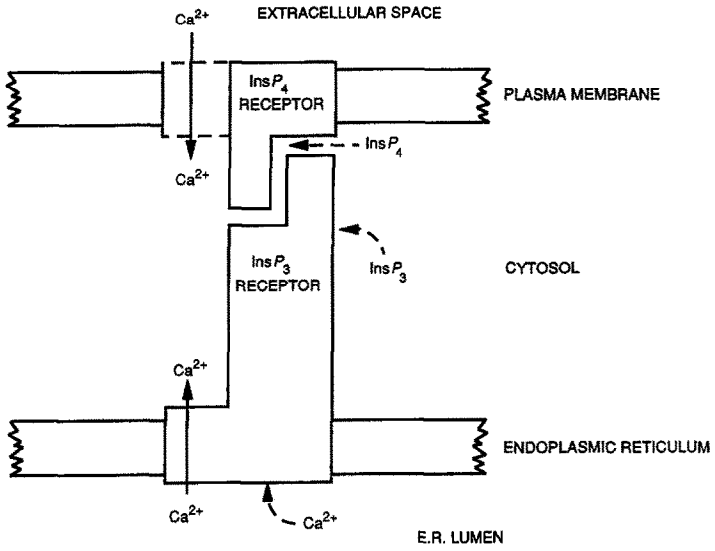

Fig. 2. Proposed interactions of inositol phosphates in regulating Ca mobilisation and entry. This is a schematic depiction of how the $InsP_3$ receptor might span the e.r./plasma membrane space when the two membranes are in close apposition, in a manner analogous to the ryanodine receptor bridging the gap between the sarcoplasmic reticulum and the sarcolemma [9], to interact with a protein in the plasma membrane (possibly the $InsP_4$ receptor). Dissociation of the two proteins results in both Ca^{2+} efflux from the e.r. through the $InsP_3$ receptor channel (assuming the receptor is in an 'active' conformation – see Fig. 1), and also influx through the plasma membrane by an unknown mechanism.

brane, and if most of these do not have $InsP_4$ receptors, then these can be mobilized by $InsP_3$ alone with no requirement for $InsP_4$. Thus it follows that the degree of synergism in intracellular Ca^{2+} mobilization observed experimentally between $InsP_3$ and $InsP_4$ will vary considerably between tissues (e.g. [22,31,32]). More generally and more importantly, it is clear from the literature on Ca^{2+} entry discussed above that, depending to a large extent on the tissue and the experimental protocol, all 3 out of (i) the Ca^{2+} content of intracellular pools, (ii) $InsP_3$ and (iii) $InsP_4$ can contribute to the control of Ca^{2+} entry [14–25,30], but their relative importance is extraordinarily variable. An input of all 3 of these components into the equilibria governing the dissociation of the two inositol phosphate receptors, as proposed here, might explain why this is so.

In conclusion, it should be noted that this present form of the proposed model is probably oversimplified. For example, one experimental observation not readily accommodated is the apparent re-uptake of Ca^{2+} induced in a permeabilised hepatoma cell line by $InsP_4$ [33]. Using the mechanism in Fig. 1, this would mean that the $InsP_3$ receptor equilibrium is shifted to the A (and C) forms so that the Ca^{2+} pump can raise intraluminal Ca^{2+} to a higher steady-state, but why $InsP_4$ should shift the equilibrium this way under these conditions, is not immediately obvious. This particular phenomenon shows a dependence on the order of addition of the inositol phosphates [33] and that in turn implies that there may be a complexity in the processes described simply here, such that they are not always freely reversible. Evidence from intact hepatocytes [34] has also implied a stimulated Ca^{2+} efflux mediated by inositol phosphates, and if the Ca^{2+} entry mechanism controlled by $InsP_4$ is a carrier rather than a channel it is possible that its polarity could be reversed under some circumstances. Finally, free Ca^{2+} in the cytoplasm can influence $InsP_3$ binding (e.g. [35,36]), so there must be other modulatory factors to be considered in some systems. Nevertheless, the proposal outlined here, which derives directly from the proposed mechanism for 'quantal' Ca^{2+} release, provides, even in this simplest form, an explanation of most of the conflicting data on inositol phosphate-induced Ca^{2+} entry, and may serve as a starting point for more detailed consideration of the dual regulation [31] of intracellular Ca^{2+} by $InsP_3$ and $InsP_4$.

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