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Topical Review

Calcium and Inositol Trisphosphate Receptors

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Introduction

It is almost twenty years since the link between hormone effects on phosphoinositide and Ca^{2+} metabolism was first proposed [55] and more than a decade since inositol 1,4,5-trisphosphate (IP_3) was shown to release Ca^{2+} from intracellular stores [7, 90]. More recently, there has been a rapid growth in our understanding of the means whereby IP_3 stimulates Ca^{2+} mobilization and initiates more complex Ca^{2+} signals [4, 76]. The receptors to which IP_3 binds, initially identified by structure-activity [12] and radioligand-binding [68, 88] studies, have been purified [92], functionally reconstituted into both lipid vesicles [23] and bilayers from which single channel events have been resolved [102], and the primary structures of IP_3 receptors have been deduced from cDNA cloning [28, 59]. These studies have revealed that IP_3 receptors are in many ways related to another family of intracellular Ca^{2+} channels, ryanodine receptors. Both families of receptors form poorly selective cation channels with conductances severalfold larger than those of Ca^{2+} channels in the plasma membrane, and each displays four similarly spaced subconductance states [86, 99, 102], an observation that may be related to their tetrameric structures. Although IP_3 and ryanodine receptors lack cation selectivity, Ca^{2+} is the only cation with a significant electrochemical gradient across the membranes of the endoplasmic and sarcoplasmic reticula; both receptors therefore behave as intracellular Ca^{2+} channels in their native setting. The two families of intracellular Ca^{2+} channels also share some sequence similarity and higher level structural organization (Section 2), and several aspects of their regulation are similar (Fig. 1). Even more striking is the ability of the two receptors to fulfill rather similar roles in both amplifying the Ca^{2+} signal resulting from Ca^{2+} entry across the plasma membrane and in mediating the regenerative propagation of Ca^{2+} signals across cells as Ca^{2+} waves (*See* p. 115).

Ryanodine receptors [44, 52, 87] and their regulation by cyclic ADP ribose [29] are considered in several reviews and they will not be further discussed except to illustrate some of the remarkable parallels with IP_3 receptors. Nor does space permit discussion of the IP_3 receptors found in the plasma membranes of some cells, which differ from intracellular IP_3 receptors in their pharmacological, structural and electrophysiological properties [41, 42].

IP_3 receptors are regulated by many intracellular factors besides IP_3; these additional regulators include not only established intracellular messengers (e.g., cyclic AMP, Ca^{2+}, cyclic GMP), but also redox potential, ATP, Mg^{2+} and pH [98]. IP_3 receptors are thus endowed with the ability to integrate different signals and transduce them into changes in cytosolic $[Ca^{2+}]$ (Fig. 1). These integrative properties, which undoubtedly differ in their detail between the various IP_3 receptor subtypes, are likely to be important. Firstly, by transducing diverse inputs into a common intracellular signalling currency, Ca^{2+}, IP_3 receptors allow cells to fully exploit the many distinctive advantages that come from the digital coding [77] of spatially organized [6] intracellular Ca^{2+} signals, benefits that may not be available to all intracellular messenger molecules. Secondly, such integration can occur only if both signals are received within an appropriate time scale; this allows IP_3 receptors to behave as

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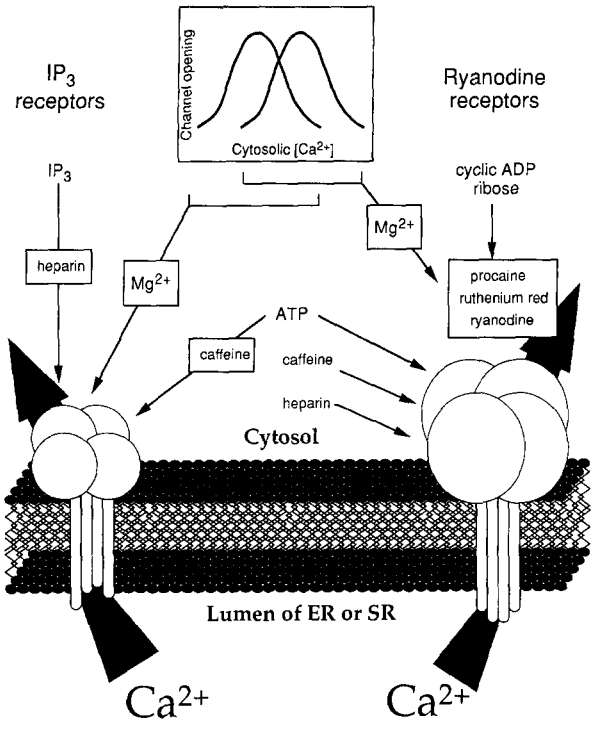

Fig. 1. IP_3 and ryanodine receptors share many functional features. Some of the more important agonists (continuous lines) and inhibitors (boxes) of IP_3 and ryanodine receptors are shown. The details differ between various subtypes of each receptor; ryanodine receptors from heart, for example, are more sensitive to cytosolic Ca^{2+} than those from skeletal muscle.

"coincidence detectors" [5] responding only if a second stimulus is received within the lifespan of the influence of the first signal. Since that lifespan may be brief, reflecting rapid dissociation of a regulatory ligand (e.g., ATP) or more long-lived as a consequence of covalent modification of the receptor (e.g., phosphorylation), the time course over which such temporal integration occurs can be tailored to specific needs. Finally, coincidence detection provides a means of improving the signal-to-noise ratio [2], a feature that assumes particular importance with Ca^{2+} signals, which can become regenerative. Of the many signals known to regulate IP_3 receptors [98] (Fig. 1), Ca^{2+} has, predictably, attracted the greatest attention and, after a brief consideration of the structure of IP_3 receptors, it will be the major focus of this review. More general reviews [4, 24, 98, 100], and reviews of the kinetics of IP_3-stimulated Ca^{2+} mobilization [96], the nature of the IP_3-sensitive Ca^{2+} stores [47], and the structure [60], pharmacology [68] and regulation [49] of IP_3 receptors can be found elsewhere.

IP_3 Receptors are Large, Tetrameric Proteins

Each of the three distinct types of IP_3-receptor subunits for which full length sequences are available, encodes a large protein of between 2671 and 2833 residues; they share 50–70% amino acid sequence identity and plainly comprise a discrete family of Ca^{2+} channels most closely related to ryanodine receptors (Fig. 2). In common with ryanodine receptors, they form tetrameric structures—the largest of all known ion channels, and both receptors have very large cytoplasmic N-terminal regulatory domains linked to a series of C-terminal membrane-spanning regions that form the intrinsic Ca^{2+} channel. Residues within the N-terminal 650 residues of the type 1 IP_3 receptor are the major determinants of IP_3 binding, and located within this region is one of two splice segments (SI, 15 residues). Another more complex splice segment (SIIA,B,C, comprising 23,1,16 residues) occurs in the large modulatory domain that separates the IP_3-binding site from the Ca^{2+} channel, the different splice variants differ in their pattern of expression [70] and in their phosphorylation by cyclic AMP-dependent protein kinase [17]. It is not yet known whether other IP_3 receptor types are alternatively spliced, although the cDNA sequences of both types 2 and 3 lack both the SI and SII segments [10, 48, 94, 103].

The modulatory domain, the region with the least, although still substantial, sequence similarity between receptor subtypes [103], also contains consensus sites for ATP binding and for phosphorylation by Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII), protein kinase C, cyclic AMP- and cyclic GMP-dependent protein kinases. None of the published IP_3 receptor sequences include conventional Ca^{2+}-binding motifs or calmodulin-binding sites, nor do they have the PEPEPEPEPE sequence thought to provide a high affinity Ca^{2+}-binding site in the skeletal muscle ryanodine receptor [15].

Hydrophobicity plots, mutagenesis studies and comparisons with other Ca^{2+} channels are most consistent with the presence of six membrane-spanning regions (M1–M6) within the 550 residues of the C-terminal of each of the IP_3 receptors [56, 58]. M1–M4 are poorly conserved between IP_3 receptors, whereas M5 and M6 are highly conserved; M6 has substantial (>50%) sequence identity with the putative M4 of ryanodine receptors [95]. These observations have lead Mikoshiba and his colleagues to propose that the pore may be formed by hydrophobic residues immediately preceding M6 of the IP_3 receptor. A high density of negatively charged residues of variable sequence in the region between M5 and M6 of IP_3 receptors and between M3 and M4 of ryanodine receptors may serve to concentrate cations around the pore of the channel [56, 103, 106]. Given the very different permeation and conductance properties of intracellular and plasma membrane cation channels, it is perhaps not surprising that there is no substantial sequence identity between their pore regions [103]. There is, however, a highly conserved GD/E motif at the boundary of the putative pore regions of both voltage and

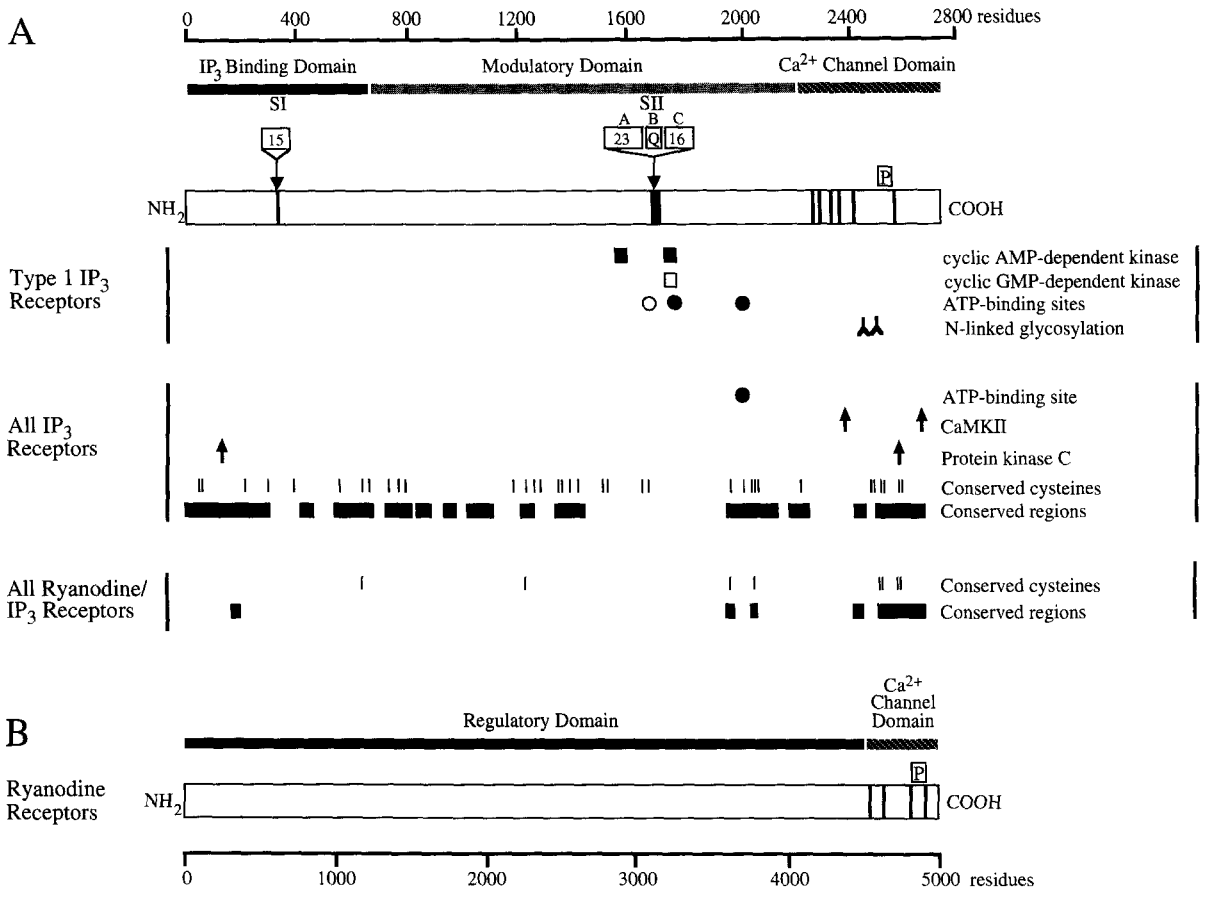

Fig. 2. Structural and functional relationships between IP_3 and ryanodine receptors. The relative sizes of each of the functional domains of IP_3 (*A*) and ryanodine (*B*) receptors are indicated by scale bars. Predicted transmembrane regions within the Ca^{2+} channel domain of each receptor are indicated by thin vertical lines and the putative pore region is indicated by [P]. The SI and SII splice segments of type 1 IP_3 receptors are indicated together with the number of residues involved (boxed) except the SIIB segment which is comprised of a single glutamine residue. Positions known to be modified in type 1 IP_3 receptors by cyclic AMP-(■) and cyclic GMP-dependent protein kinases (□), N-linked glycosylation (⅄) and putative ATP-binding sites are indicated (●; ○, site created by removal of SII segment). The putative ATP-binding site conserved in all IP_3 receptors (●) is also marked. Consensus sites for phosphorylation by CaMKII and protein kinase C conserved in all IP_3 receptors are indicated by arrows. The 35 cysteine residues conserved between all IP_3 receptors are indicated by small vertical lines and regions of >50% identity (window size = 50 residues) between all IP_3 receptors are indicated by black boxes. Similarly, the 8 cysteines conserved between all IP_3 and ryanodine receptors are indicated by small vertical lines whereas regions of >30% identity (window size = 30 residues) between all IP_3 and ryanodine receptors are indicated by black boxes.

cyclic GMP-gated cation channels that is critical in determing ion selectivity [61], and the same residues are conserved in all ryanodine and IP_3 receptors [103]. Two cytosolic and four luminal cysteine residues, which are conserved in all known ryanodine and IP_3 receptors and possibly responsible for the sensitizing effects of sulphydryl reagents [98] (Fig. 2), are also located near the proposed pore region.

Both multiple genes and alternative splicing contribute to the diversity of IP_3 receptors and the diversity will be multiplied manyfold if receptors are shown to form both heterotetramers and homotetramers [69]. Despite the diversity, each receptor appears to be built to a similar structural pattern. IP_3 binds to the extreme N-terminus to evoke a large conformational change in the receptor [58] leading to opening of the Ca^{2+} channel at the C-terminal; the large intervening modulatory domain is the target for various additional signals that serve to modulate the influence of IP_3. While tetramers of pure IP_3 [65] or ryanodine [14] receptors possess many of the features expected from their behavior *in situ*, there is evidence that accessory proteins (e.g., FK506-binding protein for ryanodine receptors and calmedin for IP_3 receptors) may be needed to confer more subtle properties [11, 39] (*See below*).

Ca^{2+} Regulates IP_3-stimulated Ca^{2+} Mobilization

Soon after the effect of IP_3 on intracellular Ca^{2+} stores was discovered, Ca^{2+} was itself shown to regulate IP_3-

stimulated Ca^{2+} mobilization. Initially, an increase in cytosolic Ca^{2+} concentration was proposed to be inhibitory [91], but it subsequently became clear that the effects of Ca^{2+} were more complex, comprising both inhibitory and stimulatory components [33, 40]. These complex effects of Ca^{2+} have attracted considerable interest [97], but it is important to stress some of the experimental difficulties that bedevil their analysis. First, the Ca^{2+} chelators (e.g., BAPTA and fura 2) used to control the $[Ca^{2+}]$ of incubation media are, in their Ca^{2+}-free forms, competitive antagonists of the IP_3 receptor [79]; changes in medium $[Ca^{2+}]$ are, therefore, inevitably accompanied by changes in the concentration of an antagonist of the IP_3 receptor. A second difficulty results from the need to examine the kinetics of both IP_3-stimulated Ca^{2+} mobilization and the regulatory effects of cytosolic Ca^{2+} on a rapid (msec) timescale. Stopped-flow techniques with fluorescent indicators [13, 53] provide the simplest approach, but since the medium $[Ca^{2+}]$ must then inevitably change, it is difficult to dissociate intrinsic properties of the IP_3 receptor from feedback regulation by Ca^{2+}. This problem is exacerbated by the observation that the free $[Ca^{2+}]$ can reach very much higher levels around open Ca^{2+} channels than can be detected by conventional, more global, measurements of cytosolic $[Ca^{2+}]$ [80, 89]. Kinetic analyses are further complicated by the curious quantal pattern of IP_3-stimulated Ca^{2+} mobilization [67, 96] and by the fact that most analyses of the effects of cytosolic Ca^{2+} are of inadequate temporal resolution to resolve the relative time courses of its stimulatory and inhibitory effects (Section 4). Thirdly, the effects of Ca^{2+} on IP_3-receptor function may sometimes have been confused by Ca^{2+}-stimulated IP_3 formation [50, 57]. A fourth problem stems from the suggestion that luminal Ca^{2+} may also regulate IP_3 receptors [37], but this effect is not universally observed and it is difficult to distinguish from regulation by the increased cytosolic $[Ca^{2+}]$ provided by Ca^{2+} leaking through open channels (*See below*). Finally, many cells express both IP_3 and ryanodine receptors and since both are regulated by cytosolic Ca^{2+}, it is important to distinguish between them; this is generally straightforward, but it is becoming clear that several of the agents (e.g., heparin and caffeine) traditionally used to distinguish between these receptors are not as selective as first thought (Fig. 1). Notwithstanding these experimental problems, there is now substantial evidence to support a widespread and perhaps ubiquitous role for cytosolic Ca^{2+} in regulating IP_3 receptors, but the problems need to be considered before ascribing significance to some of the more subtle differences in the regulatory effects of Ca^{2+} between tissues.

Biphasic Effects of Cytosolic Ca^{2+}

From bilayer recordings of cerebellar [9] and skeletal muscle [93] IP_3 receptors, rapid superfusion studies of $^{45}Ca^{2+}$-loaded brain microsomes [25], Ca^{2+} flux analyses of permeabilized hepatocytes [50], smooth muscle [33, 34], A7r5 cells [74], and various other cell lines [107], and recordings from intact *Xenopus* oocytes [104] and smooth muscle cells [35], it is clear that Ca^{2+} has both stimulatory and inhibitory effects on IP_3-stimulated Ca^{2+} mobilization. Under most experimental conditions, where receptors are exposed to stable elevated $[Ca^{2+}]$ prior to addition of IP_3, the results suggest that modest elevations of cytosolic $[Ca^{2+}]$ within the submicromolar range increase the sensitivity of IP_3 receptors to IP_3, and further, though still physiological, increases in $[Ca^{2+}]$ inhibit the response (Fig. 1). Again the parallel with ryanodine receptors is striking: just as Ca^{2+} and IP_3 coregulate IP_3 receptors, cyclic ADP ribose and Ca^{2+} coregulate at least some ryanodine receptor subtypes [29, 45]. However, whereas Ca^{2+} alone is capable of activating ryanodine receptors [44], IP_3 receptors open only in the presence of IP_3 [25, 33], and the cytosolic $[Ca^{2+}]$ ranges over which modulation occurs is substantially higher for ryanodine receptors [9], at least in the absence of cyclic ADP ribose (Fig. 1).

The kinetics of the effects of cytosolic Ca^{2+} need to be considered because while equilibrium binding of Ca^{2+} may be important in determining the influence of the resting cytosolic $[Ca^{2+}]$ in setting the sensitivity of IP_3 and ryanodine receptors, the kinetics of Ca^{2+} binding determine the behavior of the receptors as the cytosolic $[Ca^{2+}]$ rapidly changes after channel opening. Although both effects of Ca^{2+} are relatively rapid in onset (<1 sec) [25, 34, 104] and both are reversible within a few seconds [25, 50, 104], the onset of stimulation precedes inhibition. In cerebellar microsomes, where the relative time courses have been most thoroughly examined, the two effects of cytosolic Ca^{2+} are mediated by sites with similar sensitivity to Ca^{2+}, but the inhibitory effects are at least 10-fold slower in onset (time constant = 580 msec) than the stimulation [25]. Qualitative studies of other cells further support the idea that the inhibitory effect lags behind the stimulatory effect of cytosolic Ca^{2+} [34, 104]. The important point is that the effects of cytosolic $[Ca^{2+}]$ are *both* time- *and* concentration-dependent: elevations in cytosolic $[Ca^{2+}]$ that will eventually totally inhibit the IP_3 receptor, may first transiently activate it (Fig. 3). This temporal pattern of regulation by cytosolic Ca^{2+} presents yet another parallel with the behavior of ryanodine receptors, the opening of which has long been known to depend on the *rate* at which the cytosolic $[Ca^{2+}]$ increases [20, 31].

Multiple Mechanisms may Mediate the Effects of Cytosolic Ca^{2+}

Single channel recordings from skeletal muscle ryanodine receptors expressed in COS cells and then reconstituted into lipid bilayers have confirmed that the site through which cytosolic Ca^{2+} stimulates channel gating

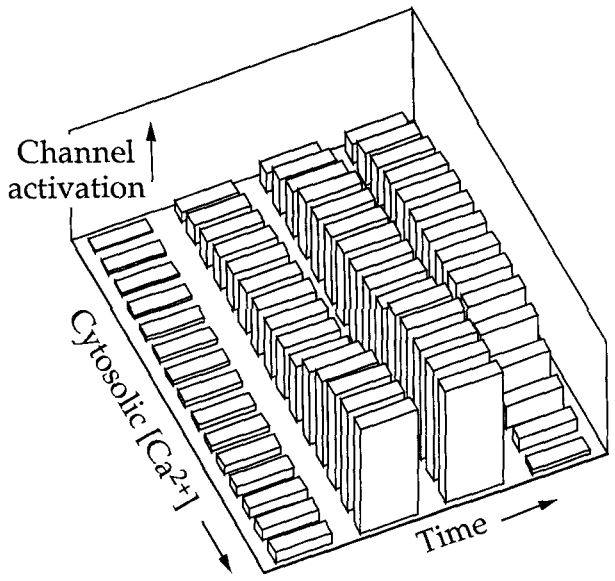

Fig. 3. The effects of cytosolic Ca^{2+} on IP_3 receptors are both time- and concentration-dependent. In this qualitative representation, the effects of cytosolic Ca^{2+} on the probability of IP_3 receptor-channel opening is shown in the continued presence of IP_3. Ca^{2+} is assumed to bind with similar affinity to two sites, a stimulatory site and an inhibitory site [25]. The onset of inhibition is substantially slower than stimulation, and occupancy of the inhibitory site inactivates the receptor irrespective of the binding of Ca^{2+} to the stimulatory site. Several important points should be noted: (i) At steady-state, cytosolic $[Ca^{2+}]$ exerts a biphasic influence on receptor activation; (ii) Maximal activation of the receptor occurs soon after addition of Ca^{2+} when the stimulatory effects dominate the slower inhibitory effect; (iii) Substantial increases in cytosolic $[Ca^{2+}]$ are first stimulatory and then inhibitory.

is intrinsic to the ryanodine receptor [14]. From studies of Ca^{2+} and ruthenium red binding to short fragments of the ryanodine receptor and the use of site-specific antibodies, the stimulatory Ca^{2+}-binding site has been mapped to a PE repeat sequence close to the first putative membrane-spanning region [15]. The mechanisms underlying the inhibitory effects of more substantial increases in cytosolic $[Ca^{2+}]$ are less thoroughly understood, although reversible phosphorylation of either the receptor or an associated protein by CaMKII has been implicated [101]. An accessory protein, namely calmodulin [45*a*], appears also to be necessary for cyclic ADP ribose to activate ryanodine receptors by increasing their sensitivity to cytosolic Ca^{2+} [45].

The mechanisms underlying the effects of cytosolic Ca^{2+} on IP_3 receptors are far less clear. Aside from the rapidity with which Ca^{2+} exerts its stimulatory effects [25] and the observation that Ca^{2+} can bind to IP_3 receptors [57], there is no direct evidence to suggest that Ca^{2+} modulates IP_3-receptor function by binding directly to the receptor. Indeed the balance of evidence suggests that accessory proteins mediate at least some of the effects of cytosolic Ca^{2+}. In cerebellum, for example, where Ca^{2+} inhibits binding to native IP_3 receptors but not to purified receptors, calmedin, a large integral membrane protein [18] mediates the inhibitory effect of cytosolic Ca^{2+}. The effects of calmedin are not restricted to brain, because it can also confer Ca^{2+}-mediated inhibition of IP_3 binding on solubilized smooth muscle IP_3 receptors [66]. A different accessory protein, possibly protein phosphatase 2B, which readily dissociates in media containing low free $[Ca^{2+}]$, is essential for the inhibitory effect of increased cytosolic $[Ca^{2+}]$ on IP_3-stimulated Ca^{2+} mobilization in cultured fibroblasts and pancreatoma cells [107, 108]. The potentiation of IP_3 responses by cytosolic Ca^{2+} in these cells has been proposed to be mediated by phosphorylation by CaMKII [107], a proposal that is consistent with a report of inhibition of IP_3-stimulated Ca^{2+} mobilization by calmodulin antagonists [32] and the observation that CaMKII stoichiometrically phosphorylates brain IP_3 receptors [22].

However, even if the IP_3 receptor is the immediate target of Ca^{2+}-regulated phosphorylation and dephosphorylation steps, it is difficult to reconcile these relatively slow enzymatic processes with the demonstrated ability of cytosolic Ca^{2+} to very rapidly modulate IP_3 receptor behavior [25, 34]. Furthermore, the regulatory effects of cytosolic Ca^{2+} are unaffected in hepatocytes by removal of ATP, by chilling, or by the presence of calmodulin antagonists [51; S. Patel and C.W. Taylor, *unpublished observation*], again suggesting that a Ca^{2+}-regulated phosphorylation-dephosphorylation cycle is unlikely to be universal. Liver, where the biphasic effects of cytosolic Ca^{2+} on IP_3 receptors are mediated by two distinct Ca^{2+}-binding sites [51], also provides a contrast with cerebellum: increased cytosolic $[Ca^{2+}]$ increases the affinity of the liver IP_3 receptor for IP_3 [50, 75], but decreases IP_3 binding in cerebellum [18]. The discrepancy may result from the much greater level of expression of calmedin in brain [66] obscuring the stimulatory effect of Ca^{2+} on IP_3 binding. Alternatively, tissues may differ in their cohorts of IP_3 receptors, in their levels of the accessory proteins that regulate their behavior, and in their levels of Ca^{2+}-regulated kinases and phosphatases.

To summarize, biphasic regulation by cytosolic Ca^{2+} is a widespread and probably universal feature of IP_3 receptors, but the mechanisms remain unclear. The rapidity of the effects would seem to necessitate a direct effect of Ca^{2+} on the IP_3 receptor, but no such effect has yet been shown. Where accessory proteins have been implicated (e.g., calmedin, CAMKII, protein phosphatase 2B), their effects do not appear to be ubiquitous. It remains possible that biphasic regulation of IP_3 receptors by cytosolic Ca^{2+} is of such profound importance in controlling Ca^{2+} mobilization (*See* p. 115) that several redundant means of achieving it have evolved.

Does Luminal Ca^{2+} Regulate IP_3 Receptors?

In an imaginative attempt to explain quantal responses to IP_3 [96], Irvine first proposed that luminal Ca^{2+} regulates

IP_3 receptors [37]. He suggested that cytosolic IP_3 and luminal Ca^{2+} were both needed to allow opening of the IP_3 receptor, and that as the luminal $[Ca^{2+}]$ fell as Ca^{2+} leaked from the stores, the sensitivity of the IP_3 receptor decreased and became insensitive to the concentration of IP_3 that first activated it. Ca^{2+} would thereby be trapped within the stores, and would be released only when either the luminal $[Ca^{2+}]$ or the cytosolic $[IP_3]$ were increased. Although the model, which has received experimental support [46, 62], provides an explanation for some aspects of quantal Ca^{2+} mobilization, it cannot accommodate more recent observations of quantal Mn^{2+} entry into empty stores [78] and quantal responses from purified receptors in vesicles without a transmembrane Ca^{2+} gradient [21]. Nor can the model be readily reconciled with the observation that appropriate concentrations of IP_3 can stimulate quantal mobilization of any fraction of the IP_3-sensitive stores [54, 62, 96], whereas the effects of luminal Ca^{2+} are evident only when stores are substantially depleted of Ca^{2+} [50, 74].

Although the proposed effects of luminal Ca^{2+} on IP_3 receptors may not provide a sufficient explanation for the curious kinetics of IP_3-stimulated Ca^{2+} mobilization, they have provoked a fresh controversy: Are the apparent effects of luminal Ca^{2+} truly mediated by Ca^{2+} binding to a site within the Ca^{2+} stores, or are they a further manifestation of the effects of cytosolic Ca^{2+} (*See* p. 112)? Before addressing this ticklish problem, we need to briefly consider the evidence, itself controversial, marshalled in support of luminal Ca^{2+} regulating the IP_3 receptor. First, stores depleted of Ca^{2+} are less sensitive to IP_3 [34, 72, 74], and stores overloaded with Ca^{2+} are more sensitive [64]. Others have contradicted these claims, but they have either examined the effects of only modest store depletion [16, 84]—the effects of store loading appear to be manifest only when stores are substantially depleted [50, 74], or by using pyrophosphate to enhance store loading, they have effectively buffered the change in luminal free $[Ca^{2+}]$ that would normally accompany Ca^{2+} efflux [83]. Second, Ca^{2+} within the stores modestly increases the binding of IP_3 to its receptor [73, 74]. Third, Ca^{2+} uptake into empty stores is insensitive to the presence of IP_3 until the stores have loaded to a critical Ca^{2+} content [72].

The possibility that modulation of responses to IP_3 by store loading may reflect secondary consequences of luminal Ca^{2+} reaching cytosolic regulatory sites is, in our view, supported by studies of A7r5 cells. In these cells, the effects of cytosolic $[Ca^{2+}]$ depend on the Ca^{2+} content of the stores: replete stores are scarcely sensitive to cytosolic $[Ca^{2+}]$, whereas stores containing very little Ca^{2+} are sensitive to modest increases in cytosolic $[Ca^{2+}]$ [63, 74]. Such similar, nonadditive effects of increased luminal and cytosolic $[Ca^{2+}]$ on IP_3 sensitivity are, of course, to be expected if the same cytosolic Ca^{2+}-binding site mediates both effects. The modest concentration of BAPTA used to control the medium $[Ca^{2+}]$ in these experiments is unlikely [74] to effectively buffer fast local free $[Ca^{2+}]$ changes in the mouths of open channels [89]. Furthermore, the observation that the effects of store-loading persist after the cytosolic $[Ca^{2+}]$ has been increased to a level that causes maximal sensitization under steady-state conditions does not exclude the possibility that the effects of luminal Ca^{2+} are mediated by cytosolic Ca^{2+} binding sites because the effects of cytosolic $[Ca^{2+}]$ are *both* time- *and* concentration-dependent (Fig. 3). It is, therefore, impossible to establish the cytosolic $[Ca^{2+}]$ at which potentiation of IP_3 responses will be maximal unless that stimulatory effect can be temporally separated from the slower inhibitory phase, otherwise the cytosolic Ca^{2+} effects reflect a balance between stimulation and inhibition, and that balance changes with time (Fig. 3). It follows that even when the cytosolic $[Ca^{2+}]$ is increased to mimic the maximal observed stimulatory effect of a sustained exposure to Ca^{2+}, Ca^{2+} rapidly leaking from replete stores may transiently further sensitize the IP_3 receptor by interacting with cytosolic Ca^{2+}-binding sites. The results from A7r5 cells do not, therefore, provide unequivocal support for a direct effect of luminal Ca^{2+} on IP_3-receptor function. Further circumstantial support in favor of the effects of luminal Ca^{2+} being mediated entirely by cytosolic Ca^{2+}-binding sites comes from the observation that the channel open time of cerebellar IP_3 receptors is much shorter when Ca^{2+} permeates from the luminal side than when either Sr^{2+} or Ba^{2+} permeate [8]. Since cytosolic Ca^{2+} is more effective than Sr^{2+} or Ba^{2+} in causing inhibition of liver IP_3 receptors [51], the single channel recordings could reflect rapid closure of channels when Ca^{2+} reaches their cytosolic surface and slower closure as Sr^{2+} and Ba^{2+} reaches the cytosol [8].

The observation that in hepatocytes, the rate of Ca^{2+} accumulation into empty stores slows in the presence of IP_3, but only after the stores have accumulated a substantial Ca^{2+} load, is consistent with a direct role for luminal Ca^{2+}. However, even this evidence, while free of the pitfalls that bedevil analyses of Ca^{2+} efflux, may simply reflect the time taken for Ca^{2+} within the store to be translocated between segregated uptake and release sites [82].

In light of the substantial difficulties, is there any evidence to directly support a role for a luminal Ca^{2+} regulatory site of the IP_3 receptor? Although the answer must presently be no, there is some evidence, although it is not conclusive, that opening of ryanodine receptors can be regulated by a luminal Ca^{2+}-binding site [71, 85]; indeed the situation is likely to be even more complex because triggers of Ca^{2+} release from SR appear to also directly stimulate dissociation of Ca^{2+} from the luminal Ca^{2+}-binding protein, calsequestrin [36]. Given, the many similarities in the behavior of IP_3 and ryanodine receptors, it would be premature, despite the lack of con-

clusive evidence, to discard the idea that luminal Ca^{2+} directly regulates IP_3 receptors. The possibility is certainly appealing since such regulation could provide a mechanism that would allow intracellular stores to refill with Ca^{2+} in the continued presence of IP_3 and might thereby contribute to the mechanisms that allow stores to reload after a Ca^{2+} spike when the transient inhibition by cytosolic Ca^{2+} has waned.

Ca^{2+} Regulation Provides Controlled Amplification

Even more striking than the shared structural and regulatory features of ryanodine and IP_3 receptors is the ability of the two receptors to fulfill rather similar roles in mediating amplification of intracellular Ca^{2+} signals. Such amplification can allow a small influx of Ca^{2+} across the plasma membrane to trigger substantial mobilization of intracellular stores, and it is an essential element of many models for regenerative propagation of Ca^{2+} waves and spikes [3, 6]. The relative roles of IP_3 and ryanodine receptors in mediating this amplification differs between tissues. In sympathetic [26] and cerebellar [19] neurones, voltage-dependent Ca^{2+} entry is amplified by Ca^{2+} release through ryanodine receptors, whereas IP_3 receptors fulfill the same role in *Xenopus* oocytes [105]. Likewise with the propagation of regenerative Ca^{2+} waves across cells: some cells (e.g., *Xenopus* oocytes) [43] rely entirely on IP_3 receptors, others entirely on ryanodine receptors (e.g., cardiac myocytes) [27], and in sea urchin eggs either ryanodine or IP_3 receptors can mediate propagation of Ca^{2+} waves [30]. These regenerative Ca^{2+} signals have been extensively reviewed [1, 3, 6, 38, 81] and will not be considered further except to stress the importance of the biphasic effects of cytosolic Ca^{2+} in allowing intracellular Ca^{2+} channels to amplify Ca^{2+} signals without sacrificing the ability to evoke graded responses.

The inextricably paired stimulatory and inhibitory effects of cytosolic Ca^{2+} on both IP_3 and ryanodine receptors and their temporal relationship (*See above*) ensure that amplification is always followed by negative feedback: the accelerator is rapidly followed by the brake. This feature prevents rampant amplification and together with the ability of IP_3 and cyclic ADP ribose to tune the sensitivity of their respective receptors to Ca^{2+} [5] allows extracellular stimuli to evoke intracellular Ca^{2+} signals that remain responsive to changes in stimulus intensity.

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