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Regulation of Ins(1,4,5) P_3 receptor isoforms by endogenous modulators

Edwin C. Thrower, Robert E. Hagar and Barbara E. Ehrlich

Three isoforms of the inositol (1,4,5)-trisphosphate [Ins(1,4,5) P_3] receptor have been identified. Each receptor isoform has been functionally characterized using many different techniques. Although these receptor isoforms possess high homology, interesting differences in their Ca^{2+} dependence, Ins(1,4,5) P_3 sensitivity and subcellular distribution exist, implying distinct cellular roles. Indeed, interplay among the isoforms might be necessary for a cell to control spatial and temporal aspects of cytosolic Ca^{2+} signals, which are important for many cellular processes. In this review isoform-specific functions, primarily at the single-channel level, will be highlighted and these properties will be correlated with Ca^{2+} signals in intact cells.

The second messenger inositol (1,4,5)-trisphosphate [Ins(1,4,5) P_3] diffuses through the cytosol and binds to Ins(1,4,5) P_3 receptors on the surface of the endoplasmic reticulum (ER), the Ca^{2+} store of the cell, where it mobilizes intracellular Ca^{2+} release.

The type I Ins(1,4,5) P_3 (IP_3 1) receptor contains >2700 amino acid residues and is highly conserved among species^{1,2}. The receptor channel is tetrameric and each subunit contains three functionally distinct regions: an Ins(1,4,5) P_3 binding domain at the N-terminal region, an ion channel pore at the C-terminal end, and a large regulatory domain separating the two regions (Fig. 1). This domain contains several putative regulatory sites¹ including those for phosphorylation (e.g. serines at positions 1589 and 1755)¹, ATP binding (residues 1773–1780 and 2016–2021)^{3,4} and Ca^{2+} binding (residues 660–745, 741–849, 994–1059, 1347–1426 and 2124–2146)⁵. In addition, there are three alternative splicing sites found in the ligand binding domain and the regulatory domain (SI, residues 318–332; SII, residues 1692–1732; and SIII,

residues 901–910)⁶. Although the functional consequences of the majority of the alternatively spliced variants are poorly understood, the SII segment is found between two cAMP-dependent protein kinase (PKA) phosphorylation sites and the presence of the SII segment determines which of these two sites is phosphorylated first⁷. There also are sites for interactions with accessory proteins, such as the Ca^{2+} binding protein calmodulin (residues 1564–1585)⁸ and the immunophilin FK506-binding protein (FKBP) (residues 1400–1401)⁹.

Two additional Ins(1,4,5) P_3 receptors, IP_3 2 and IP_3 3 receptors, have been identified^{1,6}. All three isoforms of the Ins(1,4,5) P_3 receptor are 60–70% homologous with one another¹ and vary in their tissue distribution^{1,6,10,11}. The IP_3 1 receptor is highly expressed in the CNS, particularly in the cerebellum¹. Immunohistochemistry shows that the IP_3 1 receptor is in the same cerebellar location in three mammalian species (rat, mouse and hamster) and three model non-mammalian vertebrate species (skate, claw frog and zebrafish)². Although the IP_3 2 receptor is present in many tissues, high levels are found in spinal cord and glial cells¹². The IP_3 3 receptor is found in the kidney, brain, gastrointestinal tract and pancreatic islets^{1,6,10}. The homology of Ins(1,4,5) P_3 receptor isoforms might be similar, but subtle differences in function have been observed^{13–15}. Although these differences are often modest, they have been used to explain differential signaling in cells^{11,12,14,16}.

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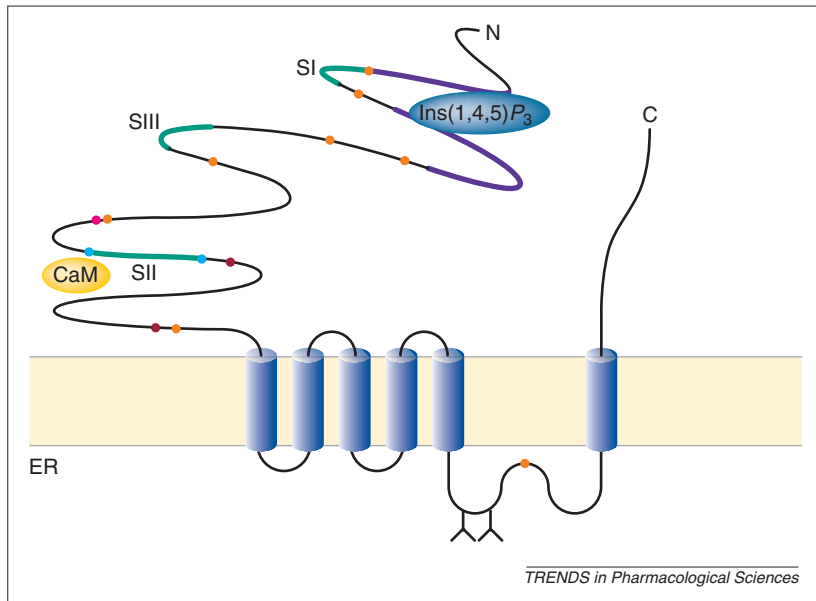


Fig. 1. Structure of the inositol (1,4,5)-trisphosphate IP_3 1 receptor. The IP_3 1 receptor is tetrameric and each subunit consists of an N-terminal ligand binding domain (purple region) followed by a regulatory domain and then a C-terminal channel domain (made up of six transmembrane-spanning regions). A molecule of inositol (1,4,5)-trisphosphate [$Ins(1,4,5)P_3$] is shown bound to the receptor. Ca^{2+} binding sites are depicted as orange circles, ATP-binding sites are shown as dark red circles, and phosphorylation sites are shown as cyan circles. Calmodulin (CaM) binds close to one of the phosphorylation sites. The site of FK506-binding protein (FKBP) binding is depicted by a pink circle. Splice regions S I, S II and S III are shown in green. Sites of N-glycosylation are also shown (Y). Abbreviation: ER, endoplasmic reticulum.

Regulation by cytoplasmic nucleotides

Cytoplasmic nucleotides, particularly ATP, can regulate $Ins(1,4,5)P_3$ receptor channel activity, but only when $Ins(1,4,5)P_3$ is present. The stimulatory effects of ATP and other cytoplasmic nucleotides on the $Ins(1,4,5)P_3$ receptor have been studied using planar lipid bilayers¹⁷ and patch clamping of *Xenopus* oocyte nuclei¹⁵. In both studies ATP was the most effective nucleotide to modulate the $Ins(1,4,5)P_3$ receptor (Fig. 2), with an ~fourfold increase in activity in the patch clamp study¹⁵ and an ~ninefold increase in the bilayer study¹⁷. In these studies, although GTP and AMP were less effective than ATP, both nucleotides produced a twofold stimulation of channel activity regardless of the technique used to study the channel (Fig. 2). Higher ATP concentrations (>0.5 mM) inhibit $Ins(1,4,5)P_3$ binding¹⁸ and Ca^{2+} release^{19,20}, an effect that can be overcome by raising the $Ins(1,4,5)P_3$ concentration^{17,21} as previously found in intact cells and binding studies^{4,18,20,22}. Thus, the inhibitory effect of ATP involves ATP competing with $Ins(1,4,5)P_3$ for the $Ins(1,4,5)P_3$ binding site, whereas activation induced by ATP occurs when ATP binds to its own binding site on the receptor.

The effects of ATP on both IP_3 1 and IP_3 3, but not IP_3 2, receptors have been studied further^{14,23}. Using controlled proteolysis and photoaffinity labeling, two ATP binding sites were found on the IP_3 1 receptor (Fig. 1) whereas only one site was found on the IP_3 3 receptor (residues 1920–1925)²³. The two ATP binding sites of the IP_3 1 receptor have affinities of 17 μ M and ~0.5 mM (Ref. 4), whereas the ATP binding site on the

IP_3 3 receptor has an affinity of 177 μ M (Ref. 23). An effect of the different affinities of these ATP binding sites is observed in A7r5 cells, which contain a 3:1 ratio of IP_3 1: IP_3 3 receptors. These cells are less sensitive to ATP stimulation compared with preparations from rat cerebellum, which contain >90% IP_3 1 receptors²³. Similarly, the rate of Ca^{2+} release in IP_3 1-receptor-expressing cells is enhanced by ATP to a greater extent than Ca^{2+} release in IP_3 3-receptor-expressing cells¹. The unequal number of ATP binding sites and the differing affinities of the two receptor isoforms could account for differences in modulation by ATP in intact cells³. An additional factor to consider is the presence of IP_3 1– IP_3 3 receptor heterotetramers, which add further diversity to ATP modulation in cells¹. Thus, regulation of $Ins(1,4,5)P_3$ -induced Ca^{2+} release by cytoplasmic nucleotides extends over a wide concentration range and varies among individual cell types.

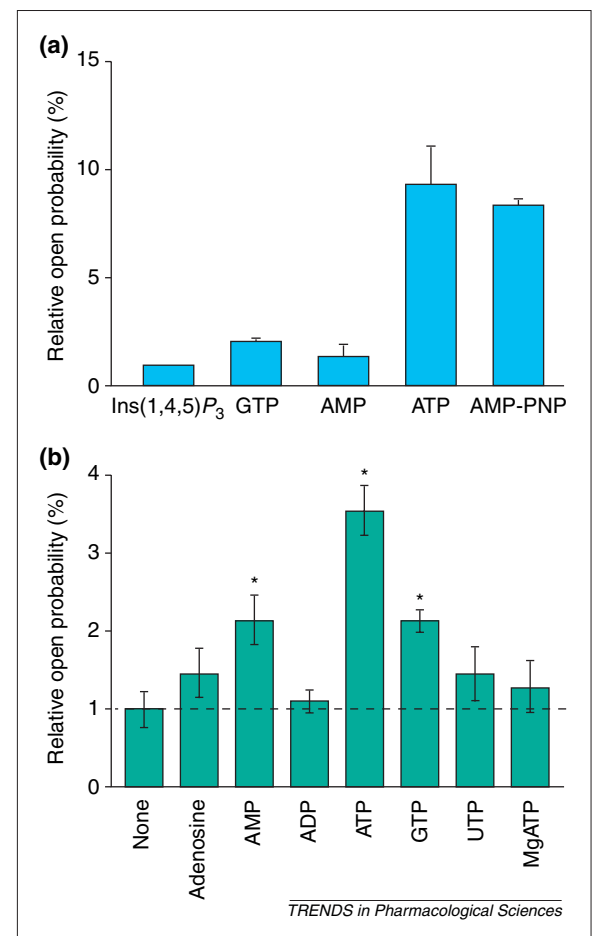


Fig. 2. Nucleotide specificity for the activation of the inositol (1,4,5)-trisphosphate IP_3 1 receptor. The relative open probability (%) of a single IP_3 1 receptor, activated by inositol (1,4,5)-trisphosphate [$Ins(1,4,5)P_3$] and measured in the presence of several different nucleotides (ATP, MgATP, ADP, AMP, adenosine, GTP or UTP) and an ATP poorly hydrolyzable analog (AMP-PNP) is shown. Two different techniques were used: (a) microsomes fused to planar lipid bilayers; and (b) *Xenopus* oocyte nuclear patches. Note the similarity in the results: ATP is the most effective nucleotide, although activation by ATP is most pronounced in planar lipid bilayer experiments. * $P < 0.05$, compared with 'none' (no nucleotide). (a) Reproduced, with permission, from Ref. 17. (b) Reproduced, with permission, from Ref. 15.

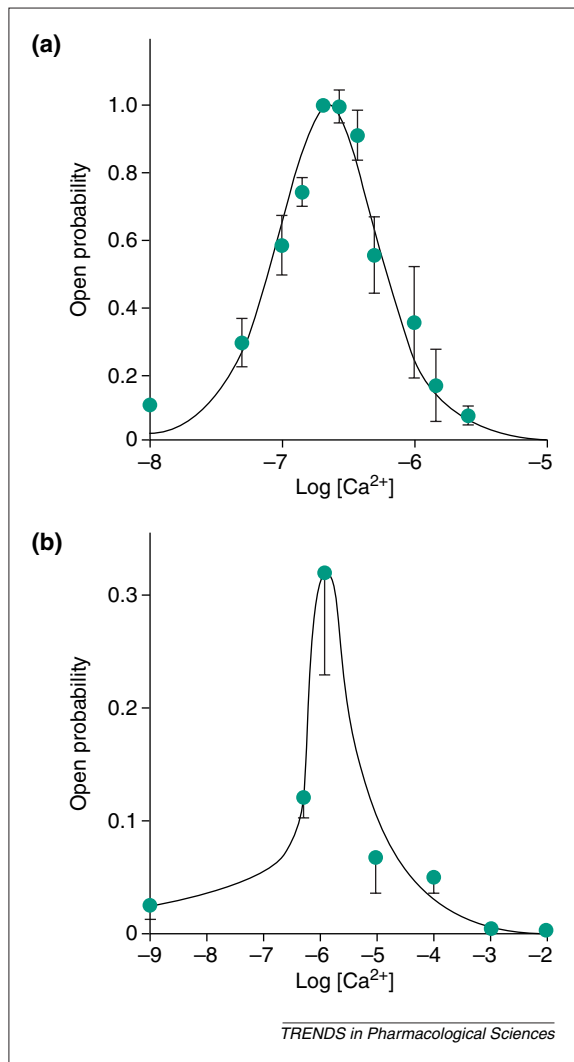


Fig. 3. Ca^{2+} dependence of the inositol (1,4,5)-trisphosphate IP_3 1 receptor. Normalized open probability is plotted as a function of the free cytoplasmic Ca^{2+} concentration for a single IP_3 1 receptor in (a) planar lipid bilayers and (b) *Xenopus* oocyte nuclear patches. Maximum IP_3 1 receptor channel activity occurs with a Ca^{2+} concentration of between 150 nM and 1 μM . (a) Reproduced, with permission, from Ref. 24. (b) Reproduced, with permission, from Ref. 25.

Regulation by cytosolic free Ca^{2+}

IP_3 1 receptors

Free Ca^{2+} has been shown to exert both stimulatory and inhibitory effects on the IP_3 1 receptor (Refs 1,6,24–31), effects that are highly concentration dependent. Small elevations in cytosolic Ca^{2+} concentrations (<300 nM) increase channel open probability; however, at higher free Ca^{2+} concentrations, inhibition of $\text{Ins}(1,4,5)\text{P}_3$ channel activity occurs^{1,32}. These results have been observed at the single-channel level with the IP_3 1 receptor in both bilayers^{1,4,13} and native nuclear patches²⁵ (Fig. 3).

The cytosolic Ca^{2+} dependency of purified IP_3 1 receptors at the single-channel level^{8,33} contrasts with results obtained in native receptors in that Ca^{2+} dependency in purified receptors does not follow a bell-shaped curve^{8,33}. Indeed, no inhibition of channel

activity was detected at cytosolic Ca^{2+} concentrations, even into the micromolar range. The discrepancy in Ca^{2+} dependence between the purified and native receptors was hypothesized to be due to an accessory protein^{33,34}. Further studies support this hypothesis by showing that the Ca^{2+} -dependent regulatory protein calmodulin can confer Ca^{2+} -dependent inactivation of the receptor⁸. In bilayer studies, addition of 10 μM calmodulin to the cytoplasmic side of the purified IP_3 1 receptor caused a significant reduction in open probability of the channel, which was activated by 0.2 μM $\text{Ins}(1,4,5)\text{P}_3$ and 200 μM cytosolic free Ca^{2+} . When the concentration of calmodulin was further increased to 20 μM complete inhibition of the channel current was observed. Ca^{2+} -dependent inactivation of native channels in cerebellar microsomes could also be reversed in the presence of calmodulin antagonists⁸. Furthermore, Ca^{2+} -dependent inhibition occurs at lower Ca^{2+} concentrations in A7r5 cells in the presence of exogenously added calmodulin³⁵. Hence, these results support the hypothesis that bell-shaped dependence on cytosolic Ca^{2+} of the IP_3 1 receptor is not an intrinsic property of this receptor, but is more likely to be mediated by calmodulin.

IP_3 2 and IP_3 3 receptors

The cytoplasmic Ca^{2+} sensitivity of single IP_3 2 and IP_3 3 receptor channels is different from that described for the IP_3 1 receptor.

Studies using native¹³ and recombinant³⁶ IP_3 2 receptors show the Ca^{2+} dependency to be sigmoidal with substantial channel activity occurring at Ca^{2+} concentrations in the micromolar range. The lack of Ca^{2+} -dependent inhibition can be explained, at least in part, by the fact that high Ca^{2+} concentrations do not inhibit $\text{Ins}(1,4,5)\text{P}_3$ binding to isolated IP_3 2 and IP_3 3 receptors whereas they do inhibit $\text{Ins}(1,4,5)\text{P}_3$ binding to the IP_3 1 receptor¹. These results are in marked contrast to the classical bell-shaped curve observed for the IP_3 1 receptor and in studies using cells expressing predominantly IP_3 2 receptors¹. A possible explanation for this apparent discrepancy could be the involvement of an accessory protein of IP_3 2 receptors that dissociates during preparation of receptors for bilayer work³⁷. Although it is possible that the accessory protein is calmodulin, its effect on IP_3 2 receptors has not yet been determined.

Single-channel studies show that the IP_3 3 receptor does not exhibit the same bell-shaped Ca^{2+} dependence curve as the IP_3 1 receptor¹⁴. However, such a lack of inhibition of the IP_3 3 receptor by Ca^{2+} has generated controversy because in other studies of recombinant IP_3 3 receptors³⁸ and in cells expressing predominantly IP_3 3 receptors³⁹ the bell-shaped Ca^{2+} dependence is observed. The reason for differences between these results and those from the bilayer studies¹⁴ is presently unknown. Even though RIN-m5F cells contain high concentrations of

calmodulin⁴⁰, it is an unlikely modulator of Ca²⁺ dependence because it does not bind to purified IP₃ receptors⁴¹. Alternatively, other accessory proteins expressed in RIN-m5F cells that confer Ca²⁺-dependent inhibition might be lost during the isolation of microsomal membranes.

Heterotetramers of the Ins(1,4,5)P₃ receptor

Although calmodulin might explain differences in the responses of Ins(1,4,5)P₃ receptor isoforms to cytoplasmic Ca²⁺, the existence of heterotetramers of the receptor isoforms might also contribute to the differing Ca²⁺ dependence of these receptors. In RIN-m5F cells, the presence of IP₃1–IP₃3 receptor heterotetramers seems unlikely because this cell line contains predominantly IP₃3 receptors (96%) with only very few IP₃1 receptors (4%) and a complete absence of IP₃2 receptors^{6,14}. However, measurable quantities of IP₃1–IP₃3 receptor heterotetramers are found in the oocyte nuclei preparation³⁸, and thus such heterotetramers might explain the bell-shaped Ca²⁺ dependence observed in this preparation. Recent evidence shows a direct association of the N-terminus of one receptor subunit with the C-terminus of an adjacent receptor subunit in heterotetrameric Ins(1,4,5)P₃ receptors⁴². This could give rise to a mechanism by which differential regulation of heterotetrameric channels could be achieved. Indeed, it has been shown that heterotetrameric Ins(1,4,5)P₃ receptors display subunit dominance with respect to regulation by Ca²⁺ and ATP, where the IP₃1 receptor is the dominant receptor subunit¹. Because heterotetramers are present, the channels should exhibit IP₃1 receptor properties, assuming that the presence of one or more IP₃1 receptor subunit(s) are sufficient to convey these characteristics to the tetrameric channel.

In conclusion, single-channel experiments analyzing the Ca²⁺ dependency of IP₃1 receptors^{1,4,8,13,25,33} and IP₃2 receptors^{13,36} are in good agreement, although differences observed between studies of IP₃3 receptors remain to be resolved^{14,21,38}. However, the mere fact that the three isoforms have different Ca²⁺ dependencies indicates the importance of Ca²⁺ regulation in determining spatial and temporal patterns of cytosolic Ca²⁺ signals for many cellular responses. Furthermore, the combination of such properties with the subcellular distribution^{11,12,14,16} of the different isoforms might provide additional mechanisms for coordinating Ca²⁺ signals within the cell.

Effect of Ins(1,4,5)P₃ on Ca²⁺ regulation

IP₃1 receptor

Although the effect of Ca²⁺ in the presence of a fixed, high concentration of Ins(1,4,5)P₃ has been considered, in the cellular environment the concentrations of Ins(1,4,5)P₃ vary. The specificities of Ins(1,4,5)P₃ receptors for inositol phosphates are similar, but they exhibit different affinities for

Ins(1,4,5)P₃ with a rank order of IP₃2 > IP₃1 > IP₃3 (Refs 1,6). Early studies of the effects of Ca²⁺ on IP₃1 receptor function were carried out at one fixed concentration of 2 μM Ins(1,4,5)P₃, a concentration that was believed to be maximal¹. When different Ins(1,4,5)P₃ concentrations were tested, the bell-shaped curve for Ca²⁺ was still apparent, but when Ins(1,4,5)P₃ concentrations were elevated dramatically, the Ca²⁺-dependent inhibition of the IP₃1 receptor was lost, a result observed in both planar lipid bilayers⁴³ and patched *Xenopus* oocyte nuclei⁴⁴. Interestingly, the amount of Ins(1,4,5)P₃ needed to observe this result differs between channels in bilayers and those in patch-clamped oocytes. The apparent discrepancy between the results obtained in oocyte nuclei and intact mammalian cells could be explained in several ways; for example, the Ins(1,4,5)P₃ receptor in frog might be different from the mammalian receptor (the deduced amino acid sequence identity is 67%)⁴⁵. Alternatively, the technique used might differentially alter a receptor-associated component.

The ability of Ins(1,4,5)P₃ to alter the Ca²⁺ dependence of Ins(1,4,5)P₃ receptors has become more relevant now that it appears that intracellular concentrations of Ins(1,4,5)P₃ are much higher than originally predicted. The concentrations of intracellular Ins(1,4,5)P₃ measured in several cell types following agonist stimulation [1–20 μM Ins(1,4,5)P₃]^{16,46–48} are often above the value frequently quoted as the K_d for Ins(1,4,5)P₃ binding to the IP₃1 receptor (50 nM). These values for intracellular Ins(1,4,5)P₃ concentrations might actually be underestimates when confined portions of the cell, such as dendrites, are considered. Thus, increasing the Ins(1,4,5)P₃ concentration can switch the self-quenching Ca²⁺ signal expected from the IP₃1 receptor to a maintained Ca²⁺ release, as observed in cells⁴⁹.

Two explanations that can account for the persistent channel activity at high Ca²⁺ and Ins(1,4,5)P₃ concentrations are the presence of a low-affinity Ins(1,4,5)P₃ binding site and a kinetic model. A low-affinity Ins(1,4,5)P₃ binding site (K_d = 10 μM), where the binding of Ins(1,4,5)P₃ to its receptor is not inhibited by Ca²⁺, has been identified for the IP₃1 receptor⁴³. Thus, the high-affinity site would predominate when Ins(1,4,5)P₃ concentrations are low in the cell. Following a large increase in the concentration of Ins(1,4,5)P₃, binding to the low-affinity site would become significant and the channel could open despite the presence of elevated cytosolic Ca²⁺ concentrations⁴³. Alternatively, channel activity could depend on the kinetics and order of binding of Ins(1,4,5)P₃ and Ca²⁺. An increased Ins(1,4,5)P₃ concentration would relieve Ca²⁺ inhibition by decreasing the affinity of Ca²⁺ for an inhibitory site on the Ins(1,4,5)P₃ receptor⁴⁴. In this scheme^{1,50}, Ins(1,4,5)P₃ must bind to the receptor first; in this state the probability of the channel opening is very

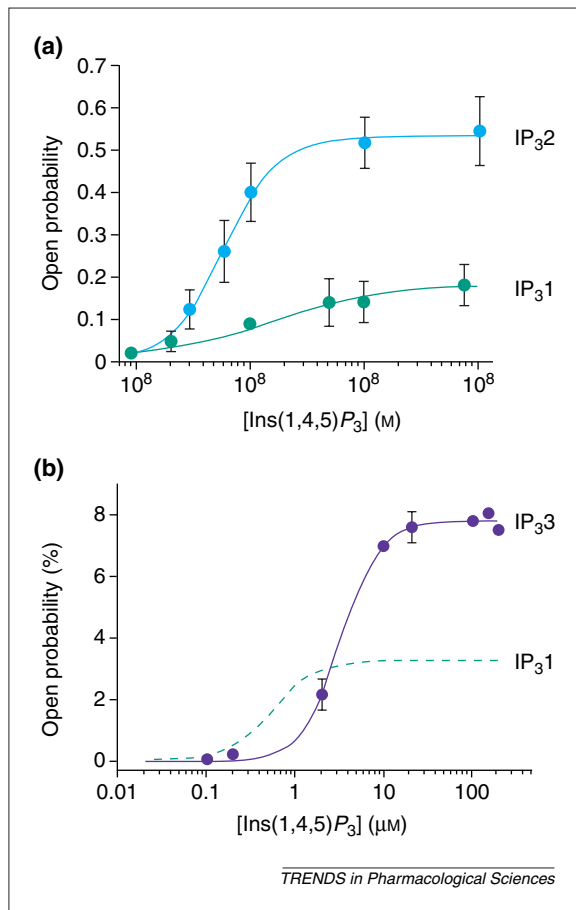


Fig. 4. (a) Inositol (1,4,5)-trisphosphate [Ins(1,4,5)P₃] dependence of the IP₃1 and IP₃2 receptor channels in planar lipid bilayers. The normalized open probability was plotted as a function of increasing Ins(1,4,5)P₃ concentration and is shown for single IP₃1 (green) and IP₃2 (cyan) receptor channels. The EC₅₀ values for IP₃1 and IP₃2 receptors were 500 nM and 28 nM, respectively. Reproduced, with permission, from Ref. 13 (the Biophysical Society, Bethesda, MD, USA). (b) Ins(1,4,5)P₃ dependence of IP₃1 (green) and IP₃3 (purple) receptor channels in planar lipid bilayers. Open probability (%) was plotted against increasing Ins(1,4,5)P₃ concentration. The IP₃3 receptor was activated at higher Ins(1,4,5)P₃ concentrations (EC₅₀ of 3200 nM versus 500 nM for IP₃1 receptors). Reproduced, with permission, from Ref. 21 (the Biophysical Society, Bethesda, MD, USA).

low. Following binding of Ins(1,4,5)P₃, the receptor can follow two paths: (1) if Ca²⁺ does not bind, it moves into an inactivated state where it cannot open; or (2) if Ca²⁺ does bind, the channel opens. Following opening of the channel, an additional binding site for Ca²⁺ is revealed and Ca²⁺ binding to this site is a requirement for Ca²⁺-dependent inactivation¹. Elevation of the Ins(1,4,5)P₃ concentration allows reactivation of the Ca²⁺-induced inactivated channel. Until the molecular mechanism for relief of Ca²⁺-dependent inhibition is clarified, however, the cellular implications for modification of the Ins(1,4,5)P₃ receptor are difficult to determine. In either case, be it a second Ins(1,4,5)P₃ binding site or order of binding of Ca²⁺ and Ins(1,4,5)P₃, high Ins(1,4,5)P₃ concentrations can maintain channel activity in the presence of high free Ca²⁺ concentrations such as during periods of prolonged stimulation.

IP₃2 and IP₃3 receptors

The Ins(1,4,5)P₃ dependency of the IP₃2 receptor^{13,36} and the IP₃3 receptor²¹ has also been investigated at the single-channel level (Fig. 4).

Both native and recombinant IP₃2 receptors^{13,36} have been studied and the IP₃2 receptor has higher Ins(1,4,5)P₃ affinity (EC₅₀ of 58 and 122 nM for native and recombinant receptors, respectively) than the IP₃1 receptor or IP₃3 receptor (EC₅₀ of 500 and 3200 nM, respectively²¹) (Fig. 4a). As stated above, the Ca²⁺ dependence of the IP₃2 receptor lacks an inactivation phase. An additional explanation might be that the IP₃2 receptor responds to lower concentrations of Ins(1,4,5)P₃ and the concentration of Ins(1,4,5)P₃ used in these measurements of channel activity is already supra-maximal. This possibility was examined further by lowering the fixed Ins(1,4,5)P₃ concentration tenfold and it was found that the Ca²⁺ dependence of the IP₃2 receptor remained sigmoidal¹³. This result clearly indicates that the interaction of Ca²⁺ and Ins(1,4,5)P₃ in regulating the single-channel behavior of the IP₃2 receptor is different from that of the IP₃1 receptor.

The open probability of the single IP₃3 receptor was examined as a function of Ins(1,4,5)P₃ concentration ranging from 0.1 to 200 μM (Ref. 21) (Fig. 4b). A model considering the effects of Ca²⁺ on Ins(1,4,5)P₃ binding predicts a leftward-shift in the steady-state open probability curve for the IP₃3 receptor as the Ins(1,4,5)P₃ concentration increases⁵¹. This was observed experimentally when a high level of IP₃3 receptor activity could be achieved at 10 nM cytosolic free Ca²⁺ and 20 μM Ins(1,4,5)P₃ (Ref. 21). Under comparable conditions, the IP₃1 receptor was inactive⁴³. Hence, it seems that high Ins(1,4,5)P₃ concentrations strongly activate the IP₃3 receptor even when cytosolic Ca²⁺ concentrations are low. This unique feature of the IP₃3 receptor, as observed at the single-channel level, would make this isoform an ideal candidate for initiating intracellular signaling events in intact cells^{11,52}.

In summary, Ins(1,4,5)P₃ interacts with each isoform in an exclusive way. In conjunction with other regulators such as Ca²⁺ and ATP, Ins(1,4,5)P₃ opens the Ins(1,4,5)P₃-gated channel in an isoform-dependent manner. These modest differences in channel function can be used in the cell to coordinate a wide spectrum of Ca²⁺ responses.

Phosphorylation–dephosphorylation of the Ins(1,4,5)P₃ receptor

Analysis of the purified cerebellar IP₃1 receptor has revealed that it can be phosphorylated by PKA, cGMP-dependent protein kinase (PKG), protein kinase C (PKC) and Ca²⁺-calmodulin-dependent protein kinase II (CaMKII)^{1,6,53}.

From the primary structure of the IP₃1 receptor, the presence of two putative consensus sequences for phosphorylation by PKA have been found in the coupling domain¹; hence, phosphorylation of the IP₃1

receptor by PKA has been the most well-characterized of all the kinases that affect IP₃1 receptors. Low PKA concentrations resulted in only one site being phosphorylated (Ser1755); however, at higher concentrations an additional site (Ser1589) was also phosphorylated⁵³. Alternative splicing of the SII segment, located between these two sites, has implications for phosphorylation of the receptor and this has been shown experimentally¹. The Ins(1,4,5)P₃ receptor from cerebellum (SII⁺, long neuronal form) and vas deferens (SII⁻, short non-neuronal form) was purified and subsequently phosphorylated by PKA. The SII⁺ receptor was phosphorylated by low PKA concentrations at Ser1755 whereas the SII⁻ form was phosphorylated almost exclusively on Ser1589 (Ref. 1). This finding might be relevant to tissue-specific regulation of the Ins(1,4,5)P₃ receptor.

The sites phosphorylated by PKA in the IP₃1 receptor are not conserved in IP₃2 and IP₃3 receptors^{1,6}, although other serines within the PKA consensus sequence are present in the coupling domains of these receptor isoforms. Such PKA consensus sequences are present at Ser1687 in rat and human IP₃2 receptors¹ and at Ser934 and Ser1133 in rat and human IP₃3 receptors, and at Ser1460 in rat IP₃3 receptors^{1,6}. PKA has been shown to phosphorylate IP₃2 and IP₃3 receptors, although with lower efficiency than for IP₃1 receptors (0.04 and 0.14 mol P_i per mol receptor, respectively, compared with 0.65 mol P_i per mol IP₃1 receptor)⁵⁴ and it enhances Ins(1,4,5)P₃-induced Ca²⁺ mobilization in a range of permeabilized cell types, irrespective of the predominant Ins(1,4,5)P₃ receptor subtype and differences in stoichiometry⁵⁴.

In pancreatic acinar cells, studies using electrophysiological methods and optical recording showed that targeting of PKA to sites of localized Ca²⁺ release conferred rapid, specific phosphoregulation of Ca²⁺ signaling⁵². Immunocytochemical evidence revealed that PKA was localized to the apical regions of acinar cells and that it co-immunoprecipitated only with IP₃3 receptors. Thus, PKA-mediated phosphorylation specifically controls a population of IP₃3 receptors that can then act as the initial trigger for Ins(1,4,5)P₃-induced Ca²⁺ release^{11,52}. Hence, when the hormone cholecystokinin (CCK) stimulates pancreatic acinar cells to secrete digestive enzymes, the Ca²⁺ oscillations induced by CCK are largely attributed to the IP₃3 receptor.

Although the focus has been on PKA, the action of PKC and CaMKII on IP₃1 receptors has been investigated, albeit to a lesser extent⁵⁵. Stimulus-induced elevation in Ca²⁺ concentrations interact with and activate PKC and CaMKII, which phosphorylates the IP₃1 receptor, further increasing Ca²⁺ flux⁵⁵. As Ca²⁺ concentrations continue to rise they also activate calmodulin and the phosphatase calcineurin. A physical association of calcineurin with the IP₃1 receptor via an immunophilin protein FKBP has been demonstrated⁵⁵. The activated calcineurin dephosphorylates the

PKC-phosphorylated site, thus decreasing Ins(1,4,5)P₃-induced Ca²⁺ flux. These findings have provided a possible molecular mechanism for closely linked phosphorylation–dephosphorylation of the Ins(1,4,5)P₃ receptor. It should be noted, however, that this is only a basic scheme, and conflicting evidence questions whether there is actually a functional interaction between IP₃1 receptors and the FKBP–calcineurin complex^{56,57}.

Regulation of the receptor by tyrosine phosphorylation has been also observed⁵⁸. During T-cell receptor stimulation the non-receptor protein tyrosine kinase, Fyn, was found to associate physically with the Ins(1,4,5)P₃ receptor. In thymocytes from *Fyn*^{-/-} mice, tyrosine phosphorylation of the receptor was reduced, coinciding with defective T-cell receptor signaling and reduced intracellular Ca²⁺ release. The fact that Fyn-mediated tyrosine phosphorylation of the Ins(1,4,5)P₃ receptor caused increased activity at higher cytosolic Ca²⁺ might indicate a shift in the Ca²⁺ dependence for inactivation. This would allow the channel to remain open throughout the sustained elevation of Ca²⁺ during T-cell activation. Thus, stimulation of the Ins(1,4,5)P₃ receptor by tyrosine phosphorylation might also play a significant role in Ca²⁺ regulation. Phosphorylation by a whole range of kinases at various serine and tyrosine residues appears to be necessary for the ‘fine-tuning’ of Ins(1,4,5)P₃ receptor regulation and might also provide a mechanism for ‘crosstalk’ between other signaling pathways⁵⁹.

Concluding remarks

Single-channel studies are ideal for looking at the basic regulatory mechanisms of the Ins(1,4,5)P₃ receptor and, along with the cellular localization, can be used to predict the form of intracellular Ca²⁺ signals. Small differences in the interactions of the endogenous regulators with each receptor isoform govern patterns of Ca²⁺ release, thus allowing fine-tuning of intracellular signals in the whole-cell environment. The IP₃1 receptor, with both Ca²⁺-dependent activation and inhibition, is an ideal candidate for establishing Ca²⁺ oscillations whereby the frequency of Ca²⁺ transients can be modulated by increasing Ins(1,4,5)P₃ concentrations. By contrast, IP₃2 receptor signals are earlier and larger, because of the greater sensitivity and efficacy of Ins(1,4,5)P₃ at mobilizing Ca²⁺ and the lack of Ca²⁺-dependent inhibition; these properties make the IP₃2 receptor a better candidate for initiation of intracellular Ca²⁺ signals at low Ins(1,4,5)P₃ concentrations rather than supporting a regenerative response. IP₃3 receptor can be activated in the presence of high Ins(1,4,5)P₃ concentrations even when the Ca²⁺ concentration is very low. This suggests that when a cell is stimulated to produce maximal levels of Ins(1,4,5)P₃, the IP₃3 receptor is responsible for initiating Ca²⁺ release. All of these similar, yet distinct, facets of the Ins(1,4,5)P₃ receptor regulation make for a rich tapestry of Ca²⁺ signaling!

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