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Review

Regulation of IP_3 receptors by cyclic AMP

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ABSTRACT

Ca^{2+} and cAMP are ubiquitous intracellular messengers and interactions between them are commonplace. Here the effects of cAMP on inositol 1,4,5-trisphosphate receptors (IP_3Rs) are briefly reviewed. All three subtypes of IP_3R are phosphorylated by cAMP-dependent protein kinase (PKA). This potentiates IP_3-evoked Ca^{2+} release through IP_3R1 and IP_3R2, but probably has little effect on IP_3R3. In addition, cAMP can directly sensitize all three IP_3R subtypes to IP_3. The high concentrations of cAMP required for this PKA-independent modulation of IP_3Rs is delivered to them within signalling junctions that include type 6 adenylyl cyclase and IP_3R2.

Contents

1. Introduction

Cyclic AMP and Ca^{2+} are ubiquitous intracellular messengers used by all eukaryotic cells from plants and animals to coordinate their behaviours in response to both extracellular signals and intracellular activity [1–3]. These messengers create a signalling 'bottleneck' through which many extracellular signals funnel to regulate diverse cellular responses. The capacity of a rather limited repertoire of intracellular messengers to selectively regulate cellular activities depends in large part on the spatial organization of the messengers within the cell, the time frames over which they are delivered, and interactions between messengers. The latter often endows signalling pathways with capacities to function as coincidence detectors: conveying signals onward only when several conditions are met [4]. As might be expected of the prototypical intracellular messengers, analyses of the interactions between cAMP and Ca^{2+} have a long history [5,6] that has revealed interactions at many levels. Ca^{2+}, for example, regulates formation and degradation of cAMP [2,7], and cAMP can regulate both the channels that allow Ca^{2+} to flow into the cytosol and the Ca^{2+} pumps that extrude it [8,9].

A ubiquitous pathway from extracellular stimuli to cytosolic Ca^{2+} signals is provided by receptors that stimulate phospholipase C (PLC), production of inositol 1,4,5-trisphosphate (IP_3) and thereby Ca^{2+} release through IP_3 receptors (IP_3R) [10]. Cyclic AMP also modulates this pathway by, for example, regulating PLC [11] and the coupling of receptors to PLC [12]. However, in this short review, I focus on just one level of interaction, that between cAMP and IP_3Rs [13,14]. IP_3R subunits are encoded by three genes in vertebrates. The three large, closely related subunits assemble into homo- and hetero-tetrameric structures, which form large-conductance Ca^{2+}-permeable channels within

Abbreviations: AC, adenylyl cyclase; EPAC, exchange protein activated by cAMP; IP_3, inositol 1,4,5-trisphosphate; IP_3R, IP_3 receptor; IRAG, IP_3R-associated cGMP kinase substrate; IRBIT, IP_3R-binding protein released by IP_3; PKA, protein kinase A (cAMP-dependent protein kinase); PKG, protein kinase G (cGMP-dependent protein kinase); PLC, phospholipase C; P_o, single-channel open probability; PTH, parathyroid hormone.

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intracellular membranes, primarily those of the endoplasmic reticulum [10]. Opening of the central pore is initiated by binding of IP_3 to all four IP_3R subunits [15], which evokes conformational changes within the N-terminal domains of the IP_3R [16]. These conformational changes are proposed to facilitate binding of Ca^{2+}, which then triggers opening of the pore. Hence, the IP_3R is itself a coincidence detector, responding only when provided with both cytosolic IP_3 and Ca^{2+}. High-resolution structures of the N-terminal region of an IP_3R with and without IP_3 bound [16], and cryo-electron microscopy reconstructions of the entire IP_3R in a closed state [17] have begun to reveal the workings of the IP_3R machinery. However, the mechanisms linking IP_3 binding to channel gating are not yet fully resolved. While IP_3 and Ca^{2+} are the essential regulators of IP_3R gating, many additional signals modulate IP_3R behaviour [18]. My focus on cAMP therefore provides only a rather restricted view of the capacity of IP_3Rs to integrate information provided by different signalling pathways.

2. Regulation of IP_3Rs by PKA

Cyclic AMP-dependent protein kinase (protein kinase A, PKA), exchange proteins activated by cAMP (EPACs), cyclic nucleotide-activated cation channels (CNGs), and some cyclic nucleotide phosphodiesterases (PDEs) are the major targets of cAMP in mammals. At least some of these targets regulate IP_3-evoked Ca^{2+} signalling. PKA, for example, stimulates Ca^{2+} uptake into the sarcoplasmic reticulum, and EPACs through the small G protein rap2B stimulate PLCε [11]. However, only PKA has been convincingly shown to interact directly with IP_3Rs. The three IP_3R subtypes are closely related, but each has a distinctive distribution of PKA phosphorylation sites. The many effects of cAMP within Ca^{2+} signalling pathways were sources of some confusion in the pioneering studies of IP_3R phosphorylation [19], but the consensus now is that PKA-mediated phosphorylation of IP_3R1 and IP_3R2 enhances their activity, while the functional significance of such phosphorylation for IP_3R3 is less clear [14,20].

Two residues (S^{1589} and S^{1755}) within the central cytosolic domain of IP_3R1 are phosphorylated by PKA, and their replacement by non-phosphorylatable alanine residues confirms that they are the only sites [21]. Phosphorylation of IP_3R1 by PKA or introduction of phosphomimetic residues ($S^{1589}E/S^{1755}E$) do not themselves open the channel, but they increase the open probability (P_o) of channels activated by IP_3. The increased P_o results from shortening of the gaps between bursts of channel openings and an increase in the duration of the bursts, with no obvious effect on IP_3 binding or the sensitivity to Ca^{2+} regulation [22]. Hence, phosphorylation of IP_3R1 by PKA improves the coupling of IP_3 and Ca^{2+} binding to channel gating by both stabilizing the bursting state of the IP_3R and destabilizing a prolonged closed state. An alternative splice site (S2, residues 1693–1732), which encodes 40 residues and is removed from non-neuronal IP_3R1, abuts the second phosphorylation site (S^{1755}). For the neuronal $S2^+$ form of IP_3R1, S^{1755} entirely mediates the effects of PKA, while in the peripheral $S2^-$ form both residues (S^{1589} and S^{1755}) must be phosphorylated for PKA to enhance IP_3-evoked Ca^{2+} release [23]. Effective phosphorylation and dephosphorylation of IP_3R1 are facilitated by tethering of PKA to IP_3R1 by AKAP9 (A-kinase-anchoring protein 9) [24] and of the protein phosphatase, PP1α, by IRBIT [25], AKAP9 or directly to the C-terminal tail of IP_3R1 [26].

The consensus sequences for PKA and cGMP-dependent protein kinase (PKG) are similar, such that some residues (e.g. S^{1755} in IP_3R1S2^+) are phosphorylated by either kinase. Yet in native tissues PKG and PKA often exert opposing effects on IP_3-evoked Ca^{2+} release. The difference may, at least in part, be due to expression of IRAG (IP_3R-associated cGMP kinase substrate), which blocks phosphorylation of IP_3R1 by PKA, and IRAG phosphorylated by PKG inhibits IP_3R [27]. Hence, IRAG diverts PKG from the PKA-phosphorylation sites and imposes its own inhibition. PKA also modulates the interaction of IP_3R1 with its endogenous antagonist, IRBIT (IP_3R-binding protein released by IP_3), apparently decreasing the affinity for IRBIT so that IP_3 more effectively competes for occupancy of their shared binding site on the IP_3R [28]. Hence in secretory epithelia, receptors that stimulate formation of cAMP and IP_3 synergistically stimulate release of IRBIT from IP_3Rs, and IRBIT then directly stimulates two of the ion transporters that sustain fluid transport [28].

Long before the discovery IP_3Rs, synergistic stimulation of a Ca^{2+}-sensitive K^+ channel by α_1-adrenoceptors (which stimulate PLC) and β-adrenoceptors (which stimulate formation of cAMP) in hepatocytes suggested that cAMP might enhance receptor-mediated Ca^{2+} release from intracellular stores [29]. Subsequent studies confirmed that PKA stimulates phosphorylation of hepatic IP_3Rs [30] and potentiates IP_3-evoked Ca^{2+} release [31,32]. IP_3R2, the major IP_3R subtype in hepatocytes, is phosphorylated by PKA at a single residue (Ser^{937}), although others suggest that IP_3R2 is a rather poor substrate for PKA [20]. Ser^{937} is unique to IP_3R2, but the functional consequences of the phosphorylation appear similar to those seen with IP_3R1, namely enhanced bursts of IP_3R gating [33]. Additional effects of PKA, including an increase in IP_3 binding affinity [30] and recruitment of IP_3Rs into functional Ca^{2+} stores [32], may also contribute to the effects of PKA on IP_3R2 in intact cells.

The effects of PKA on IP_3R3 have been least explored. In intact cells, IP_3R3 is phosphorylated by PKA at three sites (S^{916}, S^{934}, S^{1832}) that are unique to IP_3R3, with S^{934} being the most extensively phosphorylated [34]. But, at least in cells expressing only IP_3R3, PKA has no effect on IP_3-evoked Ca^{2+} release triggered by cell-surface receptors [34]. Whether the phosphorylation affects other aspects of IP_3R3 behaviour remain to be determined.

3. Direct regulation of IP_3Rs by cAMP

In HEK-293 cells stably expressing human type 1 receptors for parathyroid hormone (PTH), PTH stimulates formation of cAMP, but it does not alone evoke an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$). However, PTH potentiates the increase in $[Ca^{2+}]_c$ evoked by receptors that stimulate PLC, the endogenous muscarinic M_3 receptors of HEK-293 cells, for example, which can be activated by carbachol (Fig. 1A). This effect of PTH is mimicked by stimulation of endogenous prostanoid receptors or β-adrenoceptors, by direct activation of adenylyl cyclase with forskolin or by addition of a membrane-permeant analog of cAMP, 8-Br-cAMP. The non-additive effects of maximally effective concentrations of PTH and 8-Br-cAMP confirm that the effect of PTH on carbachol-evoked Ca^{2+} signals is entirely mediated by cAMP (Fig. 1B) [35,36]. Responses to other PLC-coupled receptors are also potentiated by PTH, and the enhanced responses are not associated with increased production of IP_3 [35,37]. Furthermore, cAMP also potentiates the Ca^{2+} signals evoked by a membrane-permeant form of IP_3 (IP_3-BM) [38]. These results, demonstrating that cAMP acts downstream of IP_3, are important because cAMP can, through EPACs, stimulate PLCε [11]. However, the effects of PTH are neither mimicked by EPAC-selective analogs of cAMP [36] nor blocked by an EPAC antagonist [39]. The enhanced IP_3 –evoked increases in $[Ca^{2+}]_c$ are not due inhibition of Ca^{2+} extrusion from the cytosol by cAMP [38]. Furthermore, cAMP potentiates IP_3-evoked Ca^{2+} release in permeabilized cells [40], and it enhances IP_3-gated channel activity in nuclear patch-clamp recordings of IP_3R [40]. These results, where cAMP potentiates the activation of IP_3R by IP_3, seem consistent with the many reports suggesting that phosphorylation of IP_3R1 and IP_3R2 by PKA enhances responses to IP_3 (see preceding section). However,