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Review

A primer of NAADP-mediated Ca^{2+} signalling: From sea urchin eggs to mammalian cells

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ABSTRACT

Since the discovery of the Ca^{2+} mobilizing effects of the pyridine nucleotide metabolite, nicotinic acid adenine dinucleotide phosphate (NAADP), this molecule has been demonstrated to function as a Ca^{2+} mobilizing intracellular messenger in a wide range of cell types. In this review, I will briefly summarize the distinct principles behind NAADP-mediated Ca^{2+} signalling before going on to outline the role of this messenger in the physiology of specific cell types. Central to the discussion here is the finding that NAADP principally mobilizes Ca^{2+} from acidic organelles such as lysosomes and it is this property that allows NAADP to play a unique role in intracellular Ca^{2+} signalling. Lysosomes and related organelles are small Ca^{2+} stores but importantly may also initiate a two-way dialogue with other Ca^{2+} storage organelles to amplify Ca^{2+} release, and may be strategically localized to influence localized Ca^{2+} signalling microdomains. The study of NAADP signalling has created a new and fruitful focus on the lysosome and endolysosomal system as major players in calcium signalling and pathophysiology.

1. Introduction

It is now almost twenty years since Hon Cheung Lee and his colleagues identified NAADP as the Ca^{2+} mobilizing contaminant of commercially available NADP or the product of alkali-treatment of NADP [1] that they had previously shown releases Ca^{2+} from sea urchin egg homogenates [2]. NAADP differs from NADP only in that the nicotinamide base of the latter is replaced by nicotinic acid in NAADP (Fig. 1). Although NAADP and NADP are thus molecularly very similar, this slight change in molecular structure is essential for Ca^{2+} releasing activity and implies that biological systems exhibit a very high degree of discrimination between these two molecules. This is important since endogenous cellular levels of the familiar co-enzyme NADP are some 10^4 times higher that reported for NAADP [3].

NAADP has been reported to mobilize Ca^{2+} in a very wide range of cell types. It has satisfied all the criteria of a classical second messenger [4] as generically adapted from the criteria for the first second messenger, cAMP, by Sutherland [5]. It is an endogenous molecule whose levels are modulated by extracellular stimuli or agonists added to the target cell. Exogenous NAADP mimics the actions of external stimuli, whilst antagonists block physiological effects of various stimuli. Cells and tissues express enzymes to synthesize and metabolize it. It has a demonstrable intracellular target or binding site that mediates its effects.

Many facets of NAADP mediated Ca^{2+} signalling are proving novel, not least in its target organelle, components of the endolysosomal system [6,7]. By linking cellular stimuli to the mobilization of Ca^{2+} from strategically placed organelles, NAADP as the most potent Ca^{2+} mobilizing messenger [6], has a major impact on cell regulation in a variety of systems, often acting as a trigger of Ca^{2+} signalling events [8].

Here I first establish the major principles established for NAADP as an intracellular messenger by first discussing its role in sea urchin eggs where it was first discovered and then follow by demonstrating how these principles shape its role in many of the major cells and organs in mammalian systems in health and disease.

2. Principles of NAADP signalling

Although the most recently described member of the major triumvirate of Ca^{2+} mobilizing messengers, research over the last decade or so has demonstrated that NAADP differs in many important respects to the other two major Ca^{2+} messenger molecules inositol 1,4,5 trisphosphate (IP_3) and cyclic ADP-ribose (cADPR), particularly in the details of its mode of action (Fig. 1).

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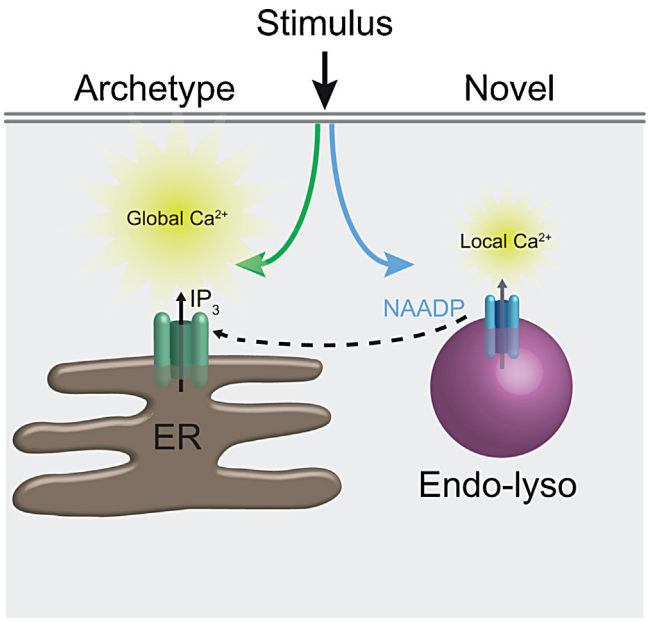

Fig. 1. Outline of the NAADP-mediated signalling pathway. The archetypical Ca^{2+} mobilization signalling pathway utilizes IP_3 as a second messenger. IP_3 is generated by receptor activation of phospholipase C which hydrolyses phosphatidylinositol 4,5 bisphosphate (PIP_2). IP_3 activates IP_3 receptors (IP_3Rs) on the endoplasmic reticulum (ER) to translocate Ca^{2+} from its lumen into the cytoplasm. NAADP is generated by stimulation of ADP-ribosyl cyclases by various receptors probably by base exchange of the nictotinamide moiety of NADP with nicotinic acid. NAADP then stimulates a small, localized Ca^{2+} release from acidic stores such as organelles of the endolysosomal system. This may provide Ca^{2+} which may act as a co-agonist with IP_3 to trigger a more global increase in cytoplasmic Ca^{2+}.

2.1. Receptor coupling to NAADP synthesis

The development of highly sensitive and selective assays to determine cellular NAADP concentrations have been important in identifying cellular stimuli coupled to NAADP synthesis [9–12]. Endogenous levels of NAADP have been widely reported and are low in resting cells [3]. A growing list of receptors including G protein-coupled receptors and tyrosine-kinase linked receptors have been shown to couple to NAADP production in a variety of cell types [6] and evoke a rapid transient increase in NAADP levels over a number of seconds.

2.2. Combinatorial signalling

It now seems likely that many stimuli and cell surface receptors coupled to Ca^{2+} signals do so but stimulating the synthesis of multiple Ca^{2+} mobilizing messengers. The relative production of the different messengers IP_3, cADPR and NAADP by activation of different cell surface receptors may produce different patterns of Ca^{2+} responses, as for example seen in pancreatic acinar cells [13]. An important theme developed here is that agonist-evoked Ca^{2+} signals are often the result of separate but interacting Ca^{2+} stores [14]. Since ER-based Ca^{2+} release channels require Ca^{2+} as a co-agonist for IP_3 [15–17] or cADPR [18,19] to open them, NAADP-evoked Ca^{2+} release from a separate organelle may provide such a trigger [8]. This may explain why many receptors known to couple to IP_3 production evoke Ca^{2+} signals that are sensitive to NAADP antagonism.

2.3. Synthesis, metabolism and transport of NAADP and paracrine effects

ADP-ribosyl cyclases, including mammalian CD38 and CD157 (BST1) are multifunctional enzymes and shown to synthesize

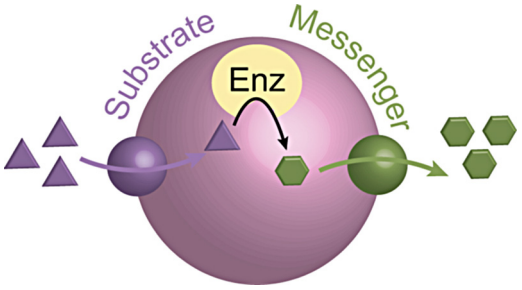

Fig. 2. Model for NAADP synthesis and regulation. ADP-ribosyl cyclases are frequently localized to the lumena of acidic organelle, and their activities are limited by substrate availability. Nucleotide transport and nicotinic acid are transported across the vesicle membrane and NAADP synthesized within the lumen. It is then translocated to the cytoplasm by different transporters. NAADP synthesis is favoured by acidic pH. It is possible that cellular stimuli regulate these transport mechanisms.

a number of pyridine nucleotide metabolites including cADPR and NAADP [20]. Certain ADP-ribosyl cyclases and CD38 have been shown to catalyze the formation of NAADP [21] by a base-exchange mechanism by which the nicotinamide moiety of NADP is exchanged for nicotinic acid, and favoured by acidic pH. This is the only reported mechanism for NAADP synthesis in biological systems despite intense investigations [22]. From studies using sea urchin sperm, a Ca^{2+}-activated NAADP synthase has been demonstrated [23], whilst In pancreatic acinar cells from *Cd38*$^{-/-}$ mice, rapid CCK stimulated NAADP is impaired [24] suggesting a role for CD38 in NAADP synthesis. However, in other cell types, receptor-stimulated NAADP synthesis persists in Cd38$^{-/-}$ cells [25,26], but the molecular identity of the synthases are unknown.

In sea urchin eggs, all isoforms of ADP-ribosyl cyclase are found inside organelles, or expressed at the plasma membrane, which presents a topological problem for messenger synthesis [27]. cADPR synthesis from NAD was suggested to occur within organelles since transport of NAD into organelles and cADPR out was found to be required for Ca^{2+} waves initiated by microinjection of NAD into the cytoplasm of intact sea urchin eggs [28]. An attractive scenario for intra-organelle synthesis of NAADP would require transport of both NADP and nicotinic acid into organelles, where an acidic environment would optimize the formation of NAADP [29] (Fig. 2). This would be followed by export of NAADP into the cytoplasm where it can stimulate Ca^{2+} release from acidic organelles. The compartmentalization of NAADP synthesis away from its cytoplasmic site of action, may mitigate against desensitization (see Section 2.6) in the sea urchin egg to low concentrations of NAADP by a bolus of NAADP efflux sufficient to activate Ca^{2+} release, and additionally NAADP could be stored as for neurotransmitters in vesicles primed for rapid release into the cytoplasm.

A number of enzyme activities have been shown to metabolize NAADP to inactive metabolites, important to terminate the signal response. In some cells, a Ca^{2+}-dependent phosphatase activity was reported which dephosphoryates NAADP to NAAD [30], as does alkaline phosphatase [31]. Alternatively, CD38 itself, as part of its multifunctional activities, has been found to degrade NAADP to ADP-ribose 2′-phosphate [29].

Several studies have shown that extracellularly applied NAADP may evoke Ca^{2+} signals in certain cell types. In some cases this is ascribed to activation of purinergic receptors at the plasma membrane coupled to various intracellular Ca^{2+} signalling pathways, including NAADP itself [32]. In others, NAADP is proposed to be transported into cells by a variety of mechanisms such as connexins and nucleotide transporters [27] where it can mobilize Ca^{2+} directly from acidic stores. In some cells, agonist-evoked intracellular elevations of NAADP levels are marked by NAADP efflux from cells [33]. Furthermore, NAADP precursors are stored in