NAADP, a new intracellular messenger that mobilizes Ca$^{2+}$ from acidic stores

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Abstract

NAADP (nicotinic acid–adenine dinucleotide phosphate) is a recently described Ca$^{2+}$-mobilizing molecule. First characterized in the sea urchin egg, it has been shown to mobilize Ca$^{2+}$ from intracellular stores in a wide range of cells from different organisms. It is a remarkably potent molecule, and recent reports show that its cellular levels change in response to a variety of agonists, confirming its role as a Ca$^{2+}$-mobilizing messenger. In many cases, NAADP appears to interact with other Ca$^{2+}$-mobilizing messengers such as IP$_3$ (inositol 1,4,5-trisphosphate) and cADPR (cADP-ribose) in shaping cytosolic Ca$^{2+}$ signals. What is not clear is the molecular nature of the NAADP-sensitive Ca$^{2+}$ release mechanism and its subcellular localization. This review focuses on the recent progress made in sea urchin eggs, which indicates that NAADP activates a novel Ca$^{2+}$ release channel distinct from the relatively well-characterized IP$_3$ and ryanodine receptors. Furthermore, in the sea urchin egg, the NAADP-sensitive store appears to be separate from the endoplasmic reticulum and is most likely an acidic store. These findings have also been reinforced by similar findings in mammalian cells, and a unified model for NAADP-induced Ca$^{2+}$ signalling is presented.

Introduction

NAADP (nicotinic acid–adenine dinucleotide phosphate) is a molecule closely related to the better-known coenzyme NADP in that it differs only by the substitution of a nicotinic acid moiety in place of nicotinamide (Figure 1). This modest difference, however, results in a dramatic change in biological activity, with the result that NAADP is a powerful Ca$^{2+}$-mobilizing agent with potencies often far exceeding those of other Ca$^{2+}$-mobilizing messengers such as IP$_3$ (inositol 1,4,5-trisphosphate) or cADPR (cADP-ribose). All the criteria for the establishment of NAADP as an intracellular Ca$^{2+}$ messenger have been satisfied. Cell-surface agonists have been found to stimulate NAADP production, cellular enzymes have been demonstrated to catalyse NAADP synthesis and metabolism, intracellular targets for NAADP have been demonstrated, and NAADP can mimic the intracellular actions of extracellular agonists, while NAADP antagonists inhibit their actions. However, several gaps remain, in particular the identification of the NAADP-sensitive Ca$^{2+}$ release mechanism and the coupling mechanisms between cell-surface receptor activation and stimulation of NAADP synthesis. In this short review, I will focus on the nature of the NAADP-sensitive Ca$^{2+}$ store and how it may participate in stimulus-response coupling.

NAADP-sensitive calcium stores

NAADP was first isolated as a contaminant of commercially available NADP, and was shown to release Ca$^{2+}$ from Percoll-purified intracellular vesicles from sea urchin eggs [1]. In this early study [1], it was noted that NAADP appeared to act on a denser fraction than the ER (endoplasmic reticulum)-derived microsomal fraction sensitive to IP$_3$. Furthermore, the NAADP-sensitive fraction could be separated from the ER by centrifugation, resulting in elongated structures. The ER and nucleus migrate to one pole of the stratified egg, while mitochondria and other dense organelles migrate to the opposite ‘pole’. If stratified eggs had previously been microinjected with a fluorescent Ca$^{2+}$ indicator and caged NAADP, cADPR or IP$_3$, then global photolysis resulted in polarized Ca$^{2+}$ transient, but photolysis of caged IP$_3$ and cADPR did not [3]. In a series of elegant experiments to visualize different Ca$^{2+}$ stores, sea urchin eggs were stratified on a sucrose cushion by centrifugation, resulting in elongated structures. The ER and nucleus migrate to one pole of the stratified egg, while mitochondria and other dense organelles migrate to the opposite ‘pole’. If stratified eggs had previously been microinjected with a fluorescent Ca$^{2+}$ indicator and caged NAADP, cADPR or IP$_3$, then global photolysis resulted in polarized Ca$^{2+}$ release, with IP$_3$ or cADPR mobilizing Ca$^{2+}$ principally from the nuclear/ER pole, while NAADP evoked a spatially segregated Ca$^{2+}$ signal at the opposite pole containing mitochondria and dense acidic...
Comparison of the structures of NADP and NAADP

NADP contains a nicotinamide moiety, while NAADP is its nicotinic acid-containing derivative. NAADP is a potent Ca\(^{2+}\)-mobilizing agent but NADP has no such activity.

**Figure 1** Comparison of the structures of NADP and NAADP

NAADP-evoked Ca\(^{2+}\) release in mammalian cells

The finding that NAADP mobilizes Ca\(^{2+}\) from acidic stores distinct from the ER in sea urchin eggs prompted investigations as to whether this is a general phenomenon in mammalian cell types too. The first intact mammalian cell shown to be responsive to NAADP was the mouse pancreatic acinar cell, which has been widely used to study fundamental mechanisms of Ca\(^{2+}\) signalling [7]. NAADP is particularly potent in these cells, with concentrations as low as 50 nM in the patch pipette evoking robust oscillatory Ca\(^{2+}\)-dependent currents, independent of extracellular Ca\(^{2+}\) [8].

Ca\(^{2+}\) imaging studies have shown that the apical pole of these epithelial cells is the most sensitive region to NAADP, away from the basolateral region containing the nucleus [9].

Calcium release in these cells in response to photolysis of caged NAADP is abolished by GPN or bafilomycin, in contrast with the effects of either IP\(_3\) or cADPR [10]. In the MIN6 pancreatic β-cell line, NAADP-evoked Ca\(^{2+}\) release is largely insensitive to thapsigargin, but is blocked by bafilomycin [10]. Rat pulmonary arterial smooth muscle cells are particularly sensitive to NAADP, with patch pipette concentrations as low as 10 nM evoking robust Ca\(^{2+}\) signals [11].

The effects of NAADP are partially resistant to thapsigargin or the ryanodine receptor blocker, ryanodine, but are completely abolished by bafilomycin. Similar results have been reported in coronary artery myocytes [12]. In the pheochromocytoma cell line, PC12, liposome-introduced NAADP induces a Ca\(^{2+}\) transient that is similarly blocked by bafilomycin and GPN [13].

A unifying feature of these studies is that NAADP-induced Ca\(^{2+}\) release may be completely blocked by treating cells with bafilomycin or GPN, consistent with an acidic store of probable lysosomal origin representing the major NAADP-sensitive organelle in these cells as well as in urchin eggs. What is variable is the degree of inhibition of NAADP-evoked Ca\(^{2+}\) by thapsigargin, ryanodine or the IP\(_3\) receptor antagonist, heparin. The ‘trigger hypothesis’ for NAADP action has been advanced to explain these results, although others have proposed a direct action on ryanodine/IP\(_3\) receptors as an alternative explanation (see [14] for a review). In its simplest terms, the trigger hypothesis states that NAADP acts on a pharmacologically distinct Ca\(^{2+}\) release channel (see [15] for a review) resident on an acidic store, with similar characteristics to lysosomes. This evokes an initial local Ca\(^{2+}\) release whose spatial localization and magnitude depend on the number and distribution of lysosomes or related organelles. Since work on NAADP-sensitive Ca\(^{2+}\) release mechanisms in broken cell preparations indicates that, in contrast with IP\(_3\) or ryanodine receptors, the NAADP-sensitive channel is not sensitive to Ca\(^{2+}\) itself and therefore cannot function as a CICR (Ca\(^{2+}\)-induced Ca\(^{2+}\) release) mechanism itself [16,17], Ca\(^{2+}\) release by NAADP is not regenerative by...
Figure 2 | Selective mobilization of separate Ca\(^{2+}\) stores by activation of different cell-surface receptors

Activation of receptors 1 and 2 mobilizes Ca\(^{2+}\) from the ER by stimulating the production of IP\(_3\) and cADPR respectively. IP\(_3\) binds and opens IP\(_3\) receptors, while cADPR is thought to modulate Ca\(^{2+}\) release via ryanodine receptors. Agonist 3 activates a receptor coupled with NAADP production. NAADP activates a distinct Ca\(^{2+}\) release mechanism on separate acidic Ca\(^{2+}\) stores, resulting in a localized Ca\(^{2+}\) signal. Ca\(^{2+}\) release via the NAADP-sensitive mechanism may also feedback onto either ryanodine receptors (dotted arrow) or IP\(_3\) receptors (not shown), resulting in a larger and more globalized Ca\(^{2+}\) release. The SERCA inhibitor, thapsigargin, empties the ER store, and abolishes responses to IP\(_3\) and cADPR as well as to agonists 1 and 2. In contrast, treatment with bafilomycin and protonophores may, by disrupting proton gradients, interfere with Ca\(^{2+}\) storage by acidic stores. This renders both NAADP and agonist 3 ineffective in terms of generating Ca\(^{2+}\) signals. While some receptors may couple with the production of one messenger, others may be coupled with more than one intracellular signalling pathway.

Itself. This was first shown in intact sea urchin eggs, where low concentrations of NAADP established Ca\(^{2+}\) gradients across the cell rather than propagated Ca\(^{2+}\) waves [18]. However, local Ca\(^{2+}\) release by NAADP may produce more globalized Ca\(^{2+}\) signals by recruiting IP\(_3\) and ryanodine receptors by promoting their role as CICR channels. Such coupling appears to be cell-specific in terms of degree and manner. In sea urchin eggs, globalized Ca\(^{2+}\) release by NAADP can occur by interactions with IP\(_3\) or ryanodine receptors, since inhibition of both is required to block the triggering of regenerative Ca\(^{2+}\) waves by NAADP [18]. In the case of pulmonary arterial myocytes, thapsigargin, ryanodine or xestospongin (an IP\(_3\) receptor blocker) prevent NAADP from producing globalized Ca\(^{2+}\) signals, but leave a residual localized Ca\(^{2+}\) release hypothesized to be the pure NAADP-evoked component [11]. The location of these local NAADP-sensitive sites correlates with regions in the cell where ryanodine receptors are closely apposed to acidic stores labelled by Lysotracker staining, and may represent trigger zones for globalizing NAADP-evoked Ca\(^{2+}\) release [19]. In pancreatic acinar cells, the case is even more extreme. Although bafilomycin or GPN selectively blocks NAADP-induced Ca\(^{2+}\) release, NAADP-evoked Ca\(^{2+}\) release is also blocked by heparin, ryanodine or the cADPR inhibitor, 8-amino-cADPR [8]. It is possible that in the apical region of these cells, the NAADP-sensitive stores are small and packed into a small volume where the Ca\(^{2+}\) buffering capacity is high, precluding the observation of pure NAADP responses in the absence of amplification by IP\(_3\) or ryanodine receptors. A different scenario is evident in MIN6 cells. Here the NAADP...
response can occur in the absence of amplification by CICR mechanisms, and thapsigargin treatment, although abolishing responses to IP_3 has little effect on NAADP-evoked Ca^{2+} release, while the reciprocal effect occurs with bafilomycin, which blocks NAADP but not IP_3-induced Ca^{2+} release [10].

In T-cell lines, there has been extensive work investigating the actions of NAADP. As in the case of pancreatic acinar cells, it has not been possible to observe effects of NAADP on Ca^{2+} release in the absence of functional ryanodine receptors [20], but the effects of bafilomycin have not been investigated here. A report at odds with results from other cells is the finding that in hepatic microsomal fractions, bafilomycin does not appear to affect Ca^{2+} release by NAADP [21]. Owing to the complexities of interactions between different Ca^{2+} stores and Ca^{2+} release channels, it is unlikely that we can gain a full understanding of NAADP-mediated Ca^{2+} signalling until the NAADP-sensitive Ca^{2+} release mechanism is identified at the molecular level.

**Receptor-mediated NAADP signalling**

There are now direct measurements of NAADP levels in a variety of cells, and NAADP production has been shown to be regulated by extracellular agonists. Receptor-mediated Ca^{2+} signalling through the NAADP signalling pathway was initially inferred from experiments in which the NAADP receptor was blocked, or by the depletion of NAADP-sensitive stores by bafilomycin or GPN. In pancreatic acinar cells, CCK (cholceystokinin), at physiological concentrations, evokes characteristic Ca^{2+} spiking, an effect that is abolished by desensitizing NAADP-sensitive Ca^{2+} release by using high concentrations of NAADP [8], or by a new range of nicotinic acid-derived pyridinium analogues that act as selective membrane-permeant inhibitors of NAADP-induced Ca^{2+} release [22]. There is specificity in this effect since both muscarinic [9] and bombesin [23] receptor-mediated signalling in these cells are not affected by such methods. Selective coupling of CCK receptors with NAADP production has been demonstrated by direct NAADP measurements, while acetylcholine had no effect on NAADP production [24]. In pulmonary artery smooth myocytes, ET-1 (endothelin-1) evokes Ca^{2+} transients that are abolished by bafilomycin and are partially resistant to thapsigargin. ET-1 has also been shown to stimulate NAADP production [19]. Prostaglandin F2α, on the other hand, evokes Ca^{2+} signals resistant to bafilomycin and does not appear to stimulate the production of NAADP. In human myometrial cells, both histamine- and oxytocin-induced Ca^{2+} transients are blocked by bafilomycin and stimulate NAADP production, while acetylcholine-induced Ca^{2+} release is unaffected by bafilomycin [25]. A recent study in human platelets also suggests the presence of multiple Ca^{2+} stores [26]. One is selectively mobilized by NAADP and recruited by thrombin receptor activation, while ADP and vasopressin mobilize a separate IP_3-sensitive pool alone [26]. These studies demonstrate the selectivity of Ca^{2+}-mobilizing agonists in terms of their differential coupling with different Ca^{2+}-mobilizing messengers. In addition, there is a good correlation between agonists that mobilize Ca^{2+} from bafilomycin-sensitive Ca^{2+} pools and those that stimulate NAADP production (Figure 2).

**Conclusions**

In summary, NAADP has now been shown to mobilize Ca^{2+} in a wide range of cell types from several mammalian and non-mammalian species. In most cells, NAADP mobilizes Ca^{2+} from a store that is distinct from the ER and most likely related to lysosomes, thus defining a new function for this organelle. NAADP levels may be modulated by several Ca^{2+}-mobilizing agonists, although the synthetic pathway for NAADP synthesis is at present poorly defined. The NAADP-sensitive Ca^{2+} release mechanism appears to be distinct from the two known major classes of Ca^{2+} release channel (IP_3 and ryanodine receptors) and differs in terms of modulation by bivalent cations and its pharmacology. In many cells, NAADP-evoked calcium release may also recruit IP_3 and ryanodine receptors, either through CICR mechanisms or possibly by direct physical interactions. The degree of coupling depends on cell type, ranging from weak coupling or independence to strong or even obligatory coupling. It is likely that the participation of multiple Ca^{2+} release mechanisms in Ca^{2+} signalling produces distinct Ca^{2+} signalling patterns, and thus allows a degree of specificity for signalling by this ubiquitous ion. For example, it has recently been shown that NAADP, but not IP_3, or cADPR, induces differentiation of PC12 cells, although all three messengers are effective at evoking Ca^{2+} signals [27]. The outstanding question in our knowledge of NAADP-mediated Ca^{2+} signalling, namely the molecular characterization of the NAADP-sensitive Ca^{2+} release mechanism, when completed will undoubtedly transform our understanding of the action of this most enigmatic of intracellular messengers.

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**References**


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