Nicotinic acid adenine dinucleotide phosphate: a new intracellular second messenger?

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THE DISCOVERY of intracellular second messengers represented a major step in understanding how extracellular signals are capable of regulating cellular behavior. In this regard, the release of intracellular calcium ions (Ca2+) plays a fundamental role in cell signaling. Release of Ca2+ from intracellular stores, such as the endoplasmic and sarcoplasmic reticulum, is a key component in several intracellular signaling pathways. Ca2+ fluxes display complex spatial and temporal signatures, enabling more information to be encoded by Ca2+ signals. To meet the demands of this complexity, cells rely on precise regulation of Ca2+ channel activity (7). Understanding of the regulation of intracellular Ca2+ release and its relationship to extracellular stimuli was greatly enhanced by the discovery of the inositol 1,4,5-trisphosphate (IP3) signaling pathway (7). In addition to IP3-induced Ca2+ release, cells contain other mechanisms for intracellular Ca2+ release (7, 23, 25, 27, 33, 35).

One of these Ca2+-releasing pathways is regulated by the newly discovered nucleotide cyclic ADP-ribose (cADPR). cADPR was discovered in 1987 by H. C. Lee and collaborators (23), who observed that incubation of sea urchin egg homogenates with nicotinamide adenine dinucleotide (NAD+) resulted in Ca2+ release from microsomal stores (23, 33). Subsequent studies revealed that the Ca2+ release activity of NAD+ was actually due to conversion of NAD+ to an active metabolite, later identified as a cyclic compound derived from the ADP-ribose moiety of NAD+ and named cADPR (39). In 1991, it was concluded that cADPR mobilizes Ca2+ by activation or sensitization of the so-called ryanodine receptor/channel (RyR) (26).

The Ca2+-releasing properties of cADPR suggested a signaling role for this molecule. Since the discovery of cADPR, much interest has been raised about the possible role of other nucleotides as second messengers involved in control of intracellular Ca2+. In fact, it was discovered that another nucleotide, nicotinic acid adenine dinucleotide phosphate (NAADP), is a potent activator of intracellular Ca2+ release (13, 36). This nucleotide activates an intracellular Ca2+ release mechanism that differs in many ways from that modulated by both IP3 and cADPR (1, 7–13, 16–18, 20–23, 25–31, 33–38). In contrast to IP3 and cADPR, the research on NAADP is only in its infancy, and further experimentation is...
needed to determine the precise role of this Ca\textsuperscript{2+}-releasing pathway in cell signaling. In this review we discuss several aspects of NAADP research and the potential role of NAADP in cellular signal transduction. NAADP has been the subject of recent descriptive reviews by other authors (27, 37, 39, 44). While briefly describing the major findings in the field, this review is devoted to a critical appraisal of several key issues that need to be resolved to determine whether NAADP is a new second messenger or a tool for the discovery of a new class of Ca\textsuperscript{2+} channels. In any case, studies of the NAADP Ca\textsuperscript{2+} release system will provide exciting new information about the complex mechanism of intracellular Ca\textsuperscript{2+} mobilization.

STRUCTURE AND DISCOVERY OF NAADP

In 1987 it was discovered that incubation of NADP in alkaline pH generated a Ca\textsuperscript{2+}-releasing metabolite (23). However, it was not until 1995 that it was described for the first time that a nicotinic acid derivative of NADP was a potent mobilizer of intracellular Ca\textsuperscript{2+} in sea urchin egg homogenates, an experimental system in which IP\textsubscript{3}-induced Ca\textsuperscript{2+} release and Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR) can be measured easily in real time (13, 36). Our laboratory analyzed the products of alkali-treated β-NADP, and, using several physicochemical methods, we (13) found that the Ca\textsuperscript{2+} releasing activity was mediated by a nucleotide with a molecular mass only 1 Da larger than β-NADP (Fig. 1). We concluded that the substance with Ca\textsuperscript{2+}-releasing properties was a NADP-related compound that has a nicotinic acid instead of a nicotinamide in the molecule. We (13) then described this molecule as NAADP. In fact, the only difference between NAADP and NADP is the change of an NH\textsubscript{2} of the amide in NADP to OH of the carboxyl group in NAADP. This substitution accounts for a difference of 1 Da between the compounds (Fig. 1).

The structural requirements of NAADP-induced Ca\textsuperscript{2+} release system appear to be very stringent, because several structural analogs of NAADP have no effect on intracellular Ca\textsuperscript{2+} release (13, 35). Of particular interest, the phosphate in position 2 is crucial for the biological activity of NAADP, because NAADP has no Ca\textsuperscript{2+}-mobilizing property (13, 35). However, changing the position of the third phosphate from 2' to 3' has no effect on the Ca\textsuperscript{2+}-releasing properties of the molecule (13, 35). In fact, whether the third phosphate is in position 2' or 3' or whether it is cyclic on positions 2' and 3' does not change the Ca\textsuperscript{2+}-mobilizing properties of this nucleotide (13, 35). Recently, a fluorescent analog of NAADP, 1,Ν\textsuperscript{6}-etheno-NAADP, with Ca\textsuperscript{2+}-mobilizing properties was synthesized (37). This compound may be a useful tool for the identification of the NAADP receptor.

UNIQUE MECHANISM OF INTRACELLULAR CA\textsuperscript{2+} RELEASE

The mechanism of Ca\textsuperscript{2+} release elicited by NAADP was initially characterized in sea urchin eggs (13, 26–31, 36, 46). In initial studies, the most striking feature of NAADP was its ability to induce Ca\textsuperscript{2+} release even after IP\textsubscript{3} and ryanodine channels had previously been desensitized (13, 36). This behavior suggested that another Ca\textsuperscript{2+} release mechanism, possibly a new Ca\textsuperscript{2+} channel, was involved in NAADP-mediated Ca\textsuperscript{2+} release. Other lines of evidence supported this notion, including findings that 1) antagonists of ryanodine and IP\textsubscript{3} channels were ineffective in blocking NAADP-mediated Ca\textsuperscript{2+} release (13); 2) known modulators of ryanodine and IP\textsubscript{3} channels, such as Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, caffeine, ryanodine, ruthenium red, and procaine as well as pH, did not influence NAADP-mediated Ca\textsuperscript{2+} release (Table 1; Refs. 11–13, 16–18, 25, 27–31, 33, 35, 36); and 3) L-type Ca\textsuperscript{2+} channel antagonists could inhibit NAADP-induced Ca\textsuperscript{2+} release but not IP\textsubscript{3}-induced Ca\textsuperscript{2+} release or CICR (28–31). Together, these findings revealed a distinct pharmacological behavior of the NAADP Ca\textsuperscript{2+} release system of sea urchin eggs, further strengthening the hypothesis that NAADP is an activator of a novel Ca\textsuperscript{2+} release system.

A remarkable distinct property of the NAADP Ca\textsuperscript{2+} release system is the self-inactivation mechanism elicited by “low” doses of NAADP: sea urchin egg homogenates preexposed to a subthreshold concentration of NAADP that does not elicit Ca\textsuperscript{2+} release per se become unresponsive to further challenges of maximal doses of NAADP (1, 28, 33). This self-inactivation mechanism is time and dose dependent, suggesting that a specific NAADP binding site is required. This behavior is also suggestive of irreversible binding of NAADP to the receptor, possibly locking the Ca\textsuperscript{2+} channel in a closed state, but this remains to be demonstrated. The inactivation mechanism might permit the NAADP Ca\textsuperscript{2+} release system to be activated in cells only once, or not at all if a low concentration of NAADP inactivates the receptor first. These characteristics suggest that the mechanism of Ca\textsuperscript{2+} release induced by NAADP may be highly tuned to detect sudden increases in NAADP concentration. Moreover, if the self-inactivation mech-
The precise role of NAADP-mediated Ca\(^{2+}\) release in sea urchin egg fertilization is not known. However, preliminary evidence indicates that NAADP-sensitive Ca\(^{2+}\) stores are activated during fertilization (46). In fact, we (46) showed that fertilization of the sea urchin egg leads to a complete inactivation of the NAADP-sensitive Ca\(^{2+}\) release. These data indicate that the Ca\(^{2+}\) pool regulated by NAADP may have an important role during sea urchin egg fertilization. NAADP-induced Ca\(^{2+}\) release has also been demonstrated in intact starfish and ascidian oocytes (47).

Plant cells. Recently, NAADP-induced Ca\(^{2+}\) release was described in cauliflower and red beet microsomes (42). However, the physiological role of this compound in plants remains unknown.

Mammalian cells. Until recently, research on NAADP-induced Ca\(^{2+}\) release was largely limited to invertebrate cells, in part because of the advantages of measuring Ca\(^{2+}\) fluxes in sea urchin preparations. More recently, NAADP-induced Ca\(^{2+}\) release has been shown to be widespread in mammalian cells and tissues (Table 2), including rat brain, T lymphocytes, vascular smooth muscle cells, cardiac myocytes, fibroblasts, and HL-60 cells (3–5, 20, 50).

It is important to note that although recent evidence indicates that mammalian cells appear to have the NAADP-responsive Ca\(^{2+}\) channel, the majority of studies have been carried out in microsomal vesicles passively loaded with Ca\(^{2+}\). In the future it will be important to determine whether NAADP can activate intracellular Ca\(^{2+}\) release in more physiological conditions. A few studies have been conducted in intact cells, however. The extensive work of Cancela and collaborators (9, 10) demonstrated that intact pancreatic acinar cells have a NAADP-responsive Ca\(^{2+}\) release system. However, the effect of NAADP on intracellular Ca\(^{2+}\) has been measured indirectly with whole cell patch clamp of Ca\(^{2+}\)-dependent currents through Cl\(^{-}\) and nonselective cation channels (9, 10). Moreover, the direct effect of NAADP on these currents is not known, and it is presumed that the effects of NAADP on the whole cell patch-clamp system are completely due to the effect of NAADP on intracellular Ca\(^{2+}\). Another important work in intact cells was conducted in human Jurkat T lymphocytes (5). Microinjection of NAADP in these cells was shown to induce a dose-dependent mobilization of intracellular Ca\(^{2+}\), which was studied with imaging using an intracellular Ca\(^{2+}\)-sensitive fluorescent dye. Akin to sea urchin eggs, NAADP-induced Ca\(^{2+}\) release in T cells displayed characteristics dis-

Table 2. Tissues and cells in which NAADP-induced Ca\(^{2+}\) release has been described

<table>
<thead>
<tr>
<th>Tissue/Cell</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea urchin egg</td>
<td>21, 22, 30, 36, 38, 46</td>
</tr>
<tr>
<td>Starfish oocytes</td>
<td>47</td>
</tr>
<tr>
<td>Ascidia oocytes</td>
<td>44</td>
</tr>
<tr>
<td>Pancreatic acinar cells</td>
<td>9, 10</td>
</tr>
<tr>
<td>Human Jurkat T lymphocytes</td>
<td>5</td>
</tr>
<tr>
<td>Homogenates and microsomes</td>
<td></td>
</tr>
<tr>
<td>Sea urchin egg homogenates</td>
<td>1, 13, 18, 28, 29, 36</td>
</tr>
<tr>
<td>Rat brain (microsomal fraction)</td>
<td>3</td>
</tr>
<tr>
<td>Rat smooth muscle cells (microsomal fraction)</td>
<td>50</td>
</tr>
<tr>
<td>Cardiac myocytes (microsomal fraction)</td>
<td>4, 50</td>
</tr>
<tr>
<td>Rat glomerular mesangial cells (microsomal fraction)</td>
<td>20, 50</td>
</tr>
<tr>
<td>HL-60 cells (microsomal fraction)</td>
<td>50</td>
</tr>
<tr>
<td>Cauliflower and red beet (microsomes)</td>
<td>42</td>
</tr>
</tbody>
</table>

It should be noted that NAADP, nicotinic acid adenine dinucleotide phosphate; cADPR, cyclic ADP-ribose; IP\(_3\), inositol 1,4,5-trisphosphate; CICR, Ca\(^{2+}\)-induced Ca\(^{2+}\)_release; [Ca\(^{2+}\)], Ca\(^{2+}\)_concentration.

Table 1. Unique properties of NAADP-induced Ca\(^{2+}\)_release system

<table>
<thead>
<tr>
<th>NAADP</th>
<th>cADPR</th>
<th>IP(_3)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium channel</td>
<td>Unknown</td>
<td>Ryanodine receptor</td>
<td>IP(<em>3)</em> receptor</td>
</tr>
<tr>
<td>Physiological agonist</td>
<td>NAADP</td>
<td>cADPR</td>
<td>IP(_3)</td>
</tr>
<tr>
<td>CICR</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Effect of low [Ca(^{2+})] (0–1 μM)</td>
<td>None</td>
<td>CICR</td>
<td>CICR</td>
</tr>
<tr>
<td>Effect of high [Ca(^{2+})] (1–100 μM)</td>
<td>None</td>
<td>Inhibitory</td>
<td>Inhibitory</td>
</tr>
<tr>
<td>Effect of Mg(^{2+}) (10 mM)</td>
<td>None</td>
<td>Inhibitory</td>
<td>Inhibitory</td>
</tr>
<tr>
<td>Sensitivity to pH (6.5–9.0)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
similar to the cADPR and IP$_3$ Ca$^{2+}$ release systems, suggesting that it may activate a unique Ca$^{2+}$ channel (5). In addition to Ca$^{2+}$ release properties, NAADP was implicated in T cell activation, because self-inactivation of the NAADP system abolished subsequent stimulation of Ca$^{2+}$ signaling via the T cell receptor-CD3 complex (5).

An important point to be considered in evaluating the role of NAADP-induced Ca$^{2+}$ release in any cell type is the validation of specificity of the NAADP effect. For instance, it is of utmost importance to document negative controls with the NAADP analogs NAAD and NADP. However, these controls appear to be lacking in many reports. Without these appropriate controls, claims of finding specific NAADP-induced Ca$^{2+}$ release should be viewed with caution.

In summary, Ca$^{2+}$ mobilization induced by NAADP has been found in both intact cells and cell-free preparations of various vertebrates and invertebrates (Table 2). Despite this progress, it remains to be established whether a physiological rather than a pharmacological NAADP signaling system is present in a widespread mode in cells. In contrast to the IP$_3$- and Ca$^{2+}$-induced Ca$^{2+}$ release systems, characterization of a ubiquitous functional NAADP signaling system is still in a very early stage.

**NAADP RECEPTOR**

The molecular identity of the NAADP receptor is still obscure. Nevertheless, specific binding of radioactive NAADP has been described in sea urchin eggs (8, 43) and in rat brain with autoradiographic techniques (45). A single and saturable binding site was more thoroughly characterized in sea urchin eggs (8, 43). In microsomes, the dissociation constant ($K_d$) was 280 pM, consistent with a high-affinity binding receptor (8). NADP and NAADPH appear to compete for binding (8, 43), but it is unclear whether this is due to NAADP present as a contaminant in NADP and NAADPH solutions used in these studies. Ca$^{2+}$ and pH did not affect NAADP binding, which probably explains why Ca$^{2+}$ and pH do not influence NAADP-induced Ca$^{2+}$ release in sea urchin eggs. More importantly, NAADP binding seemed to be irreversible (8, 44), suggesting an explanation for the molecular basis of the inactivation phenomenon observed with subthreshold concentrations of NAADP.

Furthermore, NAADP binding has also been described in brain microsomes (45), and with autoradiographic techniques those authors described that NAADP binding sites are diffusely distributed in rat brain tissues.

**SYNTHESIS AND DEGRADATION OF NAADP**

We (15, 19, 20, 41, 50) previously described synthesis of NAADP in several tissues including brain, liver, spleen, heart, and kidney glomeruli. Synthesis of NAADP can be catalyzed in vitro by a NAD(P)ase, analog to the lymphocyte antigen CD38 (2, 19), in a reaction called the base-exchange reaction (Fig. 2; Ref. 6). The enzyme catalyzes the exchange of nicotinamide for nicotinic acid on the molecule of NADP$^+$, generating NAADP (Fig. 2; Refs. 6, 13, 16, 19). Whether NAADP can be generated via the base-exchange reaction in vivo is still an open question. Under the present experimental conditions used for synthesis of NAADP, the concentrations of substrate needed, namely nicotinic acid, are several times higher than would be expected to be present in intact cells (6, 13, 19). Furthermore, the optimal pH for this reaction is out of the physiological range (2, 20). However, compartmentalization of nicotinic acid and NADP into an acid environment could theoretically provide a possible milieu for the synthesis of NAADP in vivo. Another theoretical problem is the fact that in mammalian cells, the base-exchange reaction seems to be catalyzed by CD38, which is an ectoenzyme. This therefore raises the question of how substrates would be available to the CD38 catalytic site and, once NAADP is generated, how it would be made available in the cytosol to induce Ca$^{2+}$ release.

For these reasons, it is important to consider other theoretical pathways for the synthesis of NAADP in vivo (Fig. 2). Conceivably, NAADP might be generated by deamination of NADP$^+$ (Fig. 2) or phosphorylation of NAAD$^+$. The latter is a particularly attractive hypothetical route because NAAD$^+$ is a compound present in cells and NAADP might be then catalyzed by NAD$^+$ kinase with ATP as a 2'-phosphate donor (Fig. 2). These alternative synthetic pathways ought to be explored in future studies. Notably, Lerner et al. (40) characterized a human NAD$^+$ kinase in vitro and found no evidence that it could synthesize NAADP by phosphorylation of NAAD$^+$. Nevertheless, these data do not completely exclude the possibility that NAAD$^+$ phosphorylation might perhaps occur in vivo, if intracellular cofactors or other putative physiological conditions are required to modify the enzyme and enable the reaction. They also do not exclude the possibility that other isoforms might catalyze the reaction. Therefore, the postulated NAAD$^+$ phosphorylation pathway seems unlikely at this point but cannot be completely discarded yet.

Despite the limitations discussed, the base-exchange reaction is the only pathway currently described for the synthesis of NAADP in biological systems (2, 4, 6, 13, 19, 20, 42, 50). In this regard, an important observation is that enzymes with ADP-ribosyl cyclase activity (capacity for synthesis of cyclic ADP-ribose) are also able to catalyze the synthesis of NAADP through the base-exchange reaction (2, 14, 19, 24). In fact, the mammalian version of the ADP-ribosyl cyclase (CD38) is capable of generating both NAADP and cADPR (2, 14). This observation led to the proposal of a cross talk between these two possible signaling pathways (34). However, as discussed above, whether the base-exchange reaction occurs under physiological conditions is still an open question. Using CD38 knockout mice, we (13a) determined that CD38 is the major enzyme responsible for the base-exchange reaction in mouse tissues. However, in one study (50) the capacity for synthesis of
NAADP by the base-exchange reaction in cells did not correlate with the presence of NAADP-induced Ca²⁺ release in the same cells. As result, this discrepancy raises doubts about the role of the base-exchange reaction as the physiological route for the synthesis of NAADP.

Far less is known about the pathways of NAADP degradation. NAADP hydrolysis has been described in several mammalian tissues including kidney, heart, spleen, liver, and brain. This activity appears to be mediated by the tissue alkaline phosphatase, and, in fact, isolated alkaline phosphatase is capable of NAADP hydrolysis.

**REGULATION OF NAADP SYNTHESIS**

To be considered a second messenger, the intracellular concentration of NAADP would be expected to change in response to physiological stimuli. In fact, because low concentrations of NAADP inactivate the NAADP receptor, one would expect that NAADP levels have to rise rapidly in the cytosol to activate Ca²⁺ release. Such a rapid rise in NAADP synthesis would demand a fast activation of enzymes involved in NAADP metabolism are regulated. One study in sea urchin eggs demonstrated that both cAMP and cGMP could enhance synthesis of NAADP by a membrane-bound enzyme. However, cGMP did not affect NAADP synthesis in another study. These important points need to be addressed in future studies.

Regulation of NAADP synthesis might theoretically also be upregulated by hormones or agents that increase expression of enzymes capable of catalyzing the base-exchange reaction, such as ADP-ribosyl cyclases and CD38. For example, we demonstrated that retinoic acid enhances the activity of ADP-ribosyl cyclase in rat smooth muscle and mesangial cells. Likewise, when cultured rat mesangial cells were incubated with 9-cis-retinoic acid, increased synthesis of NAADP was observed. The exact role of this and other examples of convergence of the cADPR and NAADP synthetic pathways still remains largely unexplored.

**ROLE OF NAADP IN INTRACELLULAR CA²⁺ HOMEOSTASIS**

The unique Ca²⁺-releasing properties of NAADP make it an exciting candidate for an intracellular messenger. The data obtained in sea urchin egg homoge-
nates, in which the normal architecture of intracellular Ca\(^{2+}\) stores is lost, indicate that NAADP-induced Ca\(^{2+}\) release is completely distinct and independent of the other intracellular Ca\(^{2+}\)-releasing systems regulated by cADPR and IP\(_3\) (13, 36). As discussed above, in sea urchin egg homogenates the NAADP-regulated Ca\(^{2+}\) system is not inhibited by inhibitors of the cADPR and IP\(_3\) systems. However, in intact cells the NAADP system appears to interact actively with other intracellular Ca\(^{2+}\) systems (9, 10, 21, 47). In fact, it has been proposed, for example, that in pancreatic acinar cells NAADP would be the trigger of Ca\(^{2+}\) release evoked by cholecystokinin (CCK) (9, 10) and that Ca\(^{2+}\) released by NAADP directly activates RyR, resulting in CICR, which can be amplified by further CICR.

In fact, the Ca\(^{2+}\) released from the NAADP pool can modulate the intracellular Ca\(^{2+}\) release by at least two different mechanisms: 1) a mode of priming the intracellular Ca\(^{2+}\) pools as described by Churchill and Galione (21); and 2) direct sensitization of the CICR mediated by cADPR and IP\(_3\) systems, as indicated by dual block of the cADPR and IP\(_3\) systems with 8-amino-cADPR (a cADPR antagonist) and heparin (an IP\(_3\) inhibitor). In contrast, NAADP-induced Ca\(^{2+}\) oscillations in these intact cells are insensitive to either heparin or 8-amino-cADPR alone (21, 47).

A similar role for NAADP has been proposed for the mobilization of Ca\(^{2+}\) in intact starfish oocytes and sea urchin eggs (21, 47). In these cells, microinjection or release of caged NAADP leads to a robust Ca\(^{2+}\) release followed by oscillations (21, 47). It appears that in these intact invertebrate cells NAADP-induced Ca\(^{2+}\) release can further promote Ca\(^{2+}\) mobilization by activation or sensitization of the ryanodine and IP\(_3\) receptors (21, 47). In fact, in both intact sea urchin eggs and starfish oocytes the NAADP-induced Ca\(^{2+}\) oscillations can be inhibited by dual block of the cADPR and IP\(_3\) systems with 8-amino-cADPR (a cADPR antagonist) and heparin (an IP\(_3\) inhibitor). In contrast, NAADP-induced Ca\(^{2+}\) oscillations in these intact cells are insensitive to either heparin or 8-amino-cADPR alone (21, 47).

Fig. 3. Interaction between NAADP and other Ca\(^{2+}\)-release systems. Cross talk between the NAADP Ca\(^{2+}\)-release system and the Ca\(^{2+}\)-induced Ca\(^{2+}\)-release (CICR) system may occur in 2 distinct modes. A: NAADP opens a distinct Ca\(^{2+}\)-channel resulting in Ca\(^{2+}\) release from specific intracellular stores. Released Ca\(^{2+}\) is transported by Ca\(^{2+}\)-ATPases into cyclic ADP-ribose (cADPR)-sensitive intracellular stores. These stores are then "primed" for Ca\(^{2+}\) release on activation of the ryanodine channel (RyR) by agonists such as cADPR or Ca\(^{2+}\) (CICR). B: Ca\(^{2+}\) released by NAADP directly activates RyR, resulting in CICR, which can be amplified by further CICR.
The Ca\(^{2+}\) priming of the CICR stores leads to a cycle of Ca\(^{2+}\) overload, release, and reuptake that corresponds to the Ca\(^{2+}\) oscillations (21).

In the second case, the Ca\(^{2+}\) released by NAADP could affect the apparent affinity of the RyR (9, 10). In this case, Ca\(^{2+}\) released by NAADP sensitizes the RyR to its agonists by a mechanism similar to the so-called CICR (Fig. 3B).

**NAADP Ca\(^{2+}\) POOL**

The NAADP-regulated Ca\(^{2+}\) store in sea urchin eggs is physically distinct from the cADPR and IP\(_3\) pools. In fact, two different mechanisms of intracellular Ca\(^{2+}\) uptake are observed in sea urchin egg homogenates. Sea urchin egg homogenates have both tg-sensitive and -insensitive Ca\(^{2+}\) uptake systems. These data indicate that egg homogenates have both a sarco(endo)plasmic reticulum Ca\(^{2+}\) - ATPase (SERCA)-like pool and also a second different mechanism of Ca\(^{2+}\) uptake that is not mediated by a SERCA-like enzyme. Genazzani and Galione (29) demonstrated that cADPR and IP\(_3\) promoted Ca\(^{2+}\) release only through the tg-sensitive pools. In contrast, NAADP is able to induce Ca\(^{2+}\) release from both tg-sensitive and -insensitive pools, indicating that, in sea urchin egg homogenates, the NAADP and cADPR Ca\(^{2+}\) pools are at least to some extent independent (29). More recently, it was demonstrated that the NAADP and cADPR pools can be segregated to opposite poles of intact sea urchin eggs by centrifugation (38). In addition, it was demonstrated that the NAADP-regulated Ca\(^{2+}\) pool in sea urchin eggs is distinct from the endoplasmic reticulum and mitochondria. This new, yet unidentified, Ca\(^{2+}\) pool provides exciting possibilities in NAADP research, and its identification may lead to the discovery of a new type of intracellular organelle involved in Ca\(^{2+}\) homeostasis.

**CONCLUSION—NAADP: A NEW INTRACELLULAR MESSER OR A PHARMACOLOGICAL TOOL FOR DISCOVERY OF A UNIQUE Ca\(^{2+}\) CHANNEL?**

Over the last decade, intensive research on mechanisms of intracellular calcium regulation has led to the discovery of potential new second messengers and a novel Ca\(^{2+}\) release system. The role of the NAADP system in physiological processes is being extensively investigated at the present time, and the title of this review may imply that we can provide an answer for this question. However, although NAADP displays many characteristics of a signal transduction molecule, in our opinion, we are far from answering whether NAADP is indeed an intracellular messenger.

Several requirements must be fulfilled before NAADP can be considered an intracellular messenger. 1) NAADP levels must be determined in cells. 2) The physiological pathways for the synthesis of NAADP must be defined. 3) The concentration of intracellular NAADP must be regulated by external or internal stimuli. 4) A correlation between stimulated intracellular NAADP levels and Ca\(^{2+}\) release must be established.

To date, none of these requirements has been completely fulfilled, and it would be premature to promote NAADP to the status of second messenger at this point. In fact, it is possible that NAADP may not be an intracellular messenger, and, in analogy to ryanodine, NAADP may be a pharmacological, rather than physiological, agonist of a new intracellular Ca\(^{2+}\) channel. However, even if NAADP turns out not to be a physiological agonist, it will lead to the discovery of a new class of intracellular Ca\(^{2+}\) channels with unique properties relevant to cell physiology. As discussed in this review, several pieces of the NAADP puzzle await clarification through more research investigation. Certainly, the future holds new and exciting discoveries in this field.

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