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COMMENTARY

Calcium signalling: NAADP comes out of the shadows

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The molecular mechanisms through which extracellular stimuli mobilize intracellular Ca^{2+} stores are still incompletely understood. Recent work suggests that nicotinic acid–adenine dinucleotide phosphate (NAADP) can finally join the ranks of genuine Ca^{2+}-mobilizing second messengers.

Key words: Ca^{2+} mobilization, cADP-ribose, CD38, inositol phospholipids, insulin, nicotinic acid–adenine dinucleotide phosphate (NAADP).

Although inositol 1,4,5-trisphosphate (IP_3) [1] and cADP-ribose (cADPr) [2] are now well-accepted as mediators of Ca^{2+} release from intracellular stores, the role of a third putative Ca^{2+}-mobilizing molecule, nicotinic acid–adenine dinucleotide phosphate (NAADP) [3], has until recently been more controversial. A set of five new papers [4–8], and the very recent work by Patel and co-workers [9] published in this issue of the *Biochemical Journal*, now present evidence for a role of NAADP as a *bona fide* and unique messenger molecule in a range of cell types.

NAADP is synthesized by the same enzyme family as cADPr, ADP-ribosyl cyclases, including CD38 [10]. Synthesis of NAADP involves a modification of the enzymic reaction, resulting in the exchange of nicotinamide for nicotinic acid on the unphosphorylated ribose ring of NADP [10]. The latter mechanism is favoured at low pHs such as those pertaining in an endosomal compartment where CD38 may reside during recycling from the cell surface. Replacement of an uncharged amide in NADP for a negatively charge carboxyl function in NAADP confers on the latter a potent (nanomolar affinity) capacity to mobilize Ca^{2+} from responsive stores. As first described in sea urchin egg microsomes [11], NAADP mobilizes Ca^{2+} in a number of systems from higher organisms, including mammalian T-lymphocytes [12], pancreatic acinar [13], kidney [14] and heart [15] cells.

The new papers are important because (a) they provide evidence, previously lacking, for an increase in NAADP concentration during cell stimulation, and (b) they shed light on the identity of the intracellular Ca^{2+} stores targeted by NAADP and the communication between these and other Ca^{2+} stores. Extending observations by Lee and Aarhus [16], Churchill et al. [4] used live sea urchin eggs in which the organelles are 'stratified' *in situ* by centrifugation, as well as fractionated sea urchin homogenates, to show that the NAADP-sensitive store co-segregated with a lysosomal marker and was distinct from IP_3- and cADPr-sensitive stores (which fractionated with microsomal markers). This finding explained the earlier observation [17] that the NAADP-sensitive store was insensitive to an inhibitor of the sarco(endo)plasmic reticulum Ca^{2+}-ATPases, thapsigargin. Confirming a lysosomal localization of the store targeted by NAADP, incubation of intact eggs with an agent which disrupts lysosomes significantly reduced Ca^{2+} release in response to photolysis ('uncaging') of an inactive precursor of NAADP [4].

In the context of higher organisms, microinjected NAADP was found to mobilize intracellular Ca^{2+} in single human pancreatic β-cells [5], and high concentrations of NAADP, sufficient to desensitize NAADP receptors, blocked the ability of insulin to mobilize intracellular Ca^{2+} in these cells. Correspondingly, uncaging of NAADP increased intracellular free [Ca^{2+}] in clonal β-cells [6,7], an effect unaltered by the presence of thapsigargin [7]. Importantly, increases in glucose concentration over the near-physiological range robustly increased the intracellular concentration of NAADP in clonal MIN6 β-cells and [^{32}P]NAADP bound with high affinity to a crude membrane fraction derived from these cells [6]. Strikingly, an independent study in which intra-organellar free [Ca^{2+}] was monitored with recombinant targeted probes simultaneously reports that the NAADP-sensitive store was likely to be localized principally to dense-core secretory vesicles [7]. Thus addition of NAADP to permeabilized MIN6 cells caused a significant decrease in intravesiclular free [Ca^{2+}], but had essentially no effect on endoplasmic reticulum Ca^{2+} concentrations [7]. In mice deleted in both alleles of CD38, changes in β-cell NAADP generation, and hence insulin secretion, therefore seem likely to contribute to abnormal glucose homoeostasis [18].

It seems possible that, in the case of both insulin-containing secretory vesicles and sea urchin lysosomes, the formation of spatially constrained domains of high Ca^{2+} concentration in the immediate vicinity of an individual organelle may then serve a role as an important local cue, either for the fusion of the secretory vesicle with the plasma membrane [7] or for heterotypic fusion between lysosomes and endosomes [19]. Given that dense-core secretory vesicles share a similar route of biogenesis with lysosomes [20], it is also tempting to speculate that these two organelle types may share common mechanisms for both Ca^{2+} accumulation and release. In fact, there appear to be important differences by which these two acidic organelles accumulate Ca^{2+} ions. Thus bafilomycin, an inhibitor of H^+-ATPases, blocked NAADP-induced release from the lysosome-associated store [4], but had no effect on Ca^{2+} accumulation by secretory vesicles [22]. Only in the latter was Ca^{2+} uptake blocked by vanadate, an inhibitor P-type ATPases, where silencing of the gene encoding the Ca^{2+}-ATPase, *PMR1*, also inhibited Ca^{2+} uptake [21].

An interesting possibility is that other Ca^{2+} channels, including ryanodine receptors [22], are also involved in Ca^{2+}-dependent

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amplification of the initial NAADP-mediated Ca^{2+} release from insulin-containing secretory vesicles. Such 'intra-store chatter' would complement 'inter-store' cross-talk, such as that which occurs in T-lymphocytes, where NAADP receptors are necessary for the release of Ca^{2+} via IP_3 receptors [12]. The recent work by Patel and co-workers [9] provides a direct demonstration of the latter phenomenon in neurons by showing that NAADP-sensitive stores also communicate with distinct Ca^{2+} stores at the frog neuromuscular junction. Thus NAADP applied via a liposome delivery system caused increases in intracellular [Ca^{2+}], as monitored indirectly via changes in the release of acetylcholine and miniature end-plate potentials. Importantly, NAADP-induced [Ca^{2+}] increases potentiated those evoked by the application of IP_3 or cADPr. These experiments demonstrate for the first time a Ca^{2+}-induced Ca^{2+}-release mechanism whereby Ca^{2+} liberated via NAADP receptors goes on to provoke further Ca^{2+} release via IP_3 and cADPr receptor channels.

Important questions remain regarding the role played by NAADP in intracellular Ca^{2+} signalling. Firstly, the molecular identity of the NAADP receptor remains mysterious, although the recent studies provide further convincing evidence that this is almost certainly distinct from IP_3 and cADPr receptors. Secondly, it remains unclear just how extracellular signals provoke increases in NAADP levels in target cells. In the case of glucose action on β-cells, the sensitivity of the cyclase reaction of CD38 to ATP (which favours exchange over cyclization) may be involved, since the free concentration of this nucleotide is significantly increased in β-cells following exposure to elevated glucose concentrations [23]. On the other hand, the mechanisms by which insulin might provoke (as yet unmeasured) increases in NAADP in this cell type [5] remain obscure, since insulin has no effect on free [ATP] in β-cells [24]. Likewise, the underlying mechanisms responsible for the very large increase in NAADP concentration during the acrosome reaction in sea urchin sperm [8] are unknown. Finally, the role of NAADP in the propagation of complex Ca^{2+} signals (i.e. oscillations and waves) remains to be elucidated. Although the search for answers to these questions is important, there can be little doubt that NAADP has now come of age as an important player in Ca^{2+} signalling.

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