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Review

Coupling acidic organelles with the ER through Ca^{2+} microdomains at membrane contact sites

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ABSTRACT

Acidic organelles such as lysosomes serve as non-canonical Ca^{2+} stores. The Ca^{2+} mobilising messenger NAADP is thought to trigger local Ca^{2+} release from such stores. These events are then amplified by Ca^{2+} channels on canonical ER Ca^{2+} stores to generate physiologically relevant global Ca^{2+} signals. Coupling likely occurs at microdomains formed at membrane contact sites between acidic organelles and the ER. Molecular analyses and computational modelling suggest heterogeneity in the composition of these contacts and predicted Ca^{2+} microdomain behaviour. Conversely, acidic organelles might also locally amplify and temper ER-evoked Ca^{2+} signals. Ca^{2+} microdomains between distinct Ca^{2+} stores are thus likely to be integral to the genesis of complex Ca^{2+} signals.

1. Introduction: canonical and non-canonical Ca^{2+} stores

Calcium ions perform a ubiquitous signalling role, controlling a vast array of physiological processes [1]. Ca^{2+} signals must be tightly controlled so that only appropriate processes are affected. At rest, the Ca^{2+} concentration is kept low (~100 nM), and is elevated either through Ca^{2+} influx or through release from intracellular stores. Cells achieve signal specificity by controlling both the location and timing of this increase in Ca^{2+}, with responsive proteins being tuned to specific Ca^{2+} signatures [1].

Cellular Ca^{2+} buffers limit free diffusion of the ion. Targeted Ca^{2+} release from intracellular stores represents one way to 'globalise' Ca^{2+} signals. The best characterised of these stores is the endoplasmic reticulum (ER), or sarcoplasmic reticulum (SR) in muscle. Ca^{2+} can be released from the ER/SR by intracellular second messengers such as inositol trisphosphate (IP_3) or cyclic ADP-ribose (cADPR), which act on Ca^{2+} release channels such as IP_3 receptors (IP_3Rs) or ryanodine receptors (RyRs), respectively. Sarco/Endoplasmic Reticulum Ca^{2+} ATPases (SERCA) refill the stores. Cells regulate these processes to further enhance spatiotemporal signal diversity. These mechanisms have been extensively studied since the early 1980s and are relatively well understood [1,2].

In the mid-1990s, a third Ca^{2+}-mobilising messenger was discovered [3]. Nicotinic acid adenine dinucleotide phosphate (NAADP), a contaminant in commercial stock of NADP, was shown to mobilise Ca^{2+} from cellular preparations desensitised to both IP_3 and cADPR, suggesting the existence of a novel Ca^{2+} release channel [4,5]. Moreover, the target Ca^{2+} stores also appeared novel and were later identified as a lysosome-like acidic vesicle [6,7]. Whilst the endocytic and hydrolytic functions of the endolysosomal system are well established [8], the Ca^{2+} signalling function of these organelles remained obscure until relatively recently [9]. A

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body of research has identified key physiological roles for NAADP. These include several events associated with fertilisation in invertebrate gametes [10–12], neurotransmitter release in amphibians [13] through to numerous processes in mammals such as muscle contraction [14], neurite extension [15], differentiation [16] and autophagy [17] (reviewed in [18–20]). More generally, the endolysosomal system is one of several acidic Ca^{2+} stores – a collection of morphologically distinct Ca^{2+}- and H^+-rich organelles found in all major kingdoms of life [21,22].

A number of candidates have been proposed as the molecular target for NAADP (reviewed in [23]). In 2009, papers from three independent groups converged on the two-pore channels (TPCs) as the molecular targets for NAADP [24–26]. TPCs possess a duplicated domain structure in which two voltage-gated ion channel-like domains are concatenated [27]. TPCs likely assemble as dimers [28,29], thus generating the (pseudo-) tetrameric arrangement that is characteristic of the voltage-gated ion channel superfamily, to which TPCs belong [30]. Indeed, TPCs are likely to be evolutionary intermediates between one-domain channels (such as voltage-gated K^+ channels and TRP channels) and four-domain channels (such as voltage-gated Ca^{2+} and Na^+ channels) of this superfamily [31]. Consistent with their identity as NAADP targets, TPCs localise to the endolysosomal system (reviewed in [19]). Sea urchins and most other animals possess three TPC isoforms (TPC1-3), although TPC3 appears to have undergone striking lineage-specific loss in humans and certain rodents such as mice [32,33]. NAADP function is ablated by knocking-down, knocking-out or mutating TPCs, whilst NAADP signalling is augmented by overexpressing TPCs (reviewed in [19]). Extensive subsequent research including the use of transgenic animals [25,34–37] and direct measurements of NAADP-induced TPC currents [37–43] supports the conclusion that TPCs are the targets for NAADP (reviewed in [19]). A recent report highlights a potentially important role for TPCs in Parkinson Disease, whereby chemical or molecular inhibition of TPC2 corrects lysosomal morphology defects in patient fibroblasts [44].

But controversy was never far away; a 2012 report [45] and subsequent follow-up studies [46–48] suggested that TPCs are Na^+-selective channels that may be insensitive to NAADP and instead activated by $PI(3,5)P_2$ and/or voltage. These conclusions have been questioned [49,50] and conflicting data reconciled, at least in part, by the demonstration of TPC co-regulation by NAADP and $PI(3,5)P_2$ [37,41,44]. Nonetheless, the mechanism of NAADP action on TPCs is decidedly complex. Photo-affinity labelling studies have found no evidence for a direct association between NAADP and TPCs, suggesting instead that NAADP binds low-molecular weight protein(s) in complex with TPCs [34,51,52]. These NAADP binding proteins are yet to be identified at the molecular level [53]. On balance, TPCs are likely Ca^{2+}- and Na^+-permeable channels subject to multimodal regulation.

2. The trigger hypothesis: NAADP connects Ca^{2+} stores

In the early reports of NAADP action in homogenised sea urchin eggs, NAADP activated a Ca^{2+} release mechanism distinct from either IP_3Rs or RyRs [4,54,55]. It was therefore surprising to find that NAADP-induced Ca^{2+} signals in intact mammalian cells were dependent upon functioning IP_3Rs and RyRs [6,56]. These observations were rationalised in the NAADP trigger hypothesis [20,57].

A key property of both IP_3Rs and RyRs is their biphasic regulation by Ca^{2+}. Slight elevations in Ca^{2+} open the channels and permit 'Ca^{2+}-induced Ca^{2+}-release', whereby small Ca^{2+} signals amplify themselves [58]. Conversely, high Ca^{2+} concentrations are inhibitory, thus terminating Ca^{2+} release. In contrast, NAADP-induced Ca^{2+} release is insensitive to cytosolic Ca^{2+} [59], indicating that NAADP-induced signals cannot amplify themselves. Instead, NAADP may provide a so-called 'trigger' release of Ca^{2+} that is then amplified indirectly *via* ER Ca^{2+} channels. Whilst inhibition of IP_3Rs and RyRs prevents NAADP-induced Ca^{2+} signalling in many cell types (reviewed in [60]), in general NAADP inhibition does not affect IP_3 or cADPR induced signals [56] (see Section 6). This indicates that NAADP acts 'upstream' of IP_3Rs and RyRs, consistent with a 'triggering' role. Indeed, the need for NAADP can be circumvented *in vitro* by using a lysosomotropic agent that directly mobilises Ca^{2+} from the acidic stores and generates ER-dependent Ca^{2+} signals [61]. Such data further underline an upstream role for acidic organelles and highlight that this role is independent of NAADP, avoiding complications from any potential promiscuity of NAADP action [23]. The NAADP trigger hypothesis therefore posits that small and often unresolvable NAADP-induced Ca^{2+} release events are amplified by ER receptors through Ca^{2+}-induced Ca^{2+} release (Fig. 1).

The trigger hypothesis necessitates questions about functional coupling between acidic organelles and the ER. The inability to observe NAADP-induced Ca^{2+} signals in isolation [56] would suggest a tight coupling. A close anatomical association between trigger and amplifier would explain why brief/small Ca^{2+} signals cannot be resolved by standard Ca^{2+} imaging approaches upon ER blockade in some cells. Some groups have recorded relatively low conductances for TPCs in biophysical experiments [38,39,42,43] and reports of poor Ca^{2+}-permeability [37,41,45] further indicate that the Ca^{2+} flux may be modest. If correct, such properties would probably *require* an amplification mechanism to produce global signals. Trigger and amplifier events can be dissociated upon cell homogenisation, as evidenced by the insensitivity of NAADP-induced Ca^{2+} signals in sea urchin egg homogenates to ER blockade [4,5]. Such uncoupling may also be achieved in live cells. Deleting or mutating an endolysosomal trafficking motif in the N-terminus of TPC2 redirects the channel to the plasma membrane [38]. Cells expressing the wild-type channel display fast signals that can be blocked either by inhibiting RyRs with ryanodine or by inhibiting acidification of acidic organelles with bafilomycin A_1. Cells expressing the mutant, redirected channel instead show slow, sluggish responses that are insensitive to both ryanodine and bafilomycin [38]. Close functional coupling of the acidic and ER stores therefore appears key to NAADP action.

3. Ca^{2+} microdomains at the endolysosome–ER interface: a role for membrane contact sites?

NAADP-induced Ca^{2+} signals stem from acidic organelles but are likely to be 'local' in the first instance and then amplified by the ER. This intimate functional coupling may permit the formation of Ca^{2+} microdomains at the lysosome–ER interface. Indeed, there are precedents for this. The ER is rather promiscuous in forming Ca^{2+} microdomains with both the plasma membrane and other organelles. In cardiomyocytes, Ca^{2+} influx through plasma membrane voltage-gated Ca^{2+} channels (Ca_V) generates local Ca^{2+} signals that are subsequently amplified by RyRs through Ca^{2+}-induced Ca^{2+} release (Fig. 1). This is critical for excitation–contraction coupling (see other reviews in this issue). Similarly, Ca^{2+} microdomains form between IP_3Rs on the ER and Ca^{2+} uniporters on the mitochondria. The resulting mitochondrial Ca^{2+} uptake not only tempers IP_3-evoked Ca^{2+} signals, but matches energy supply (Ca^{2+}-dependent stimulation of oxidative phosphorylation) to demand (Ca^{2+} signalling) (see other reviews in this issue) (Fig. 1). In addition, ER Ca^{2+} depletion leads to store-operated Ca^{2+} entry and store refilling through Ca^{2+} microdomains between plasma membrane Orai channels and SERCA transporters on the ER (see other reviews in this issue). Thus, analogous functional microdomains

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