Prolonged Inactivation of Nicotinic Acid Adenine Dinucleotide Phosphate-induced Ca\(^{2+}\) Release Mediates a Spatiotemporal Ca\(^{2+}\) Memory*

Grant C. Churchill‡ and Antony Galione

From the Department of Pharmacology, University of Oxford, Oxford OX1 3QT, United Kingdom

Although numerous extracellular stimuli are coupled to increases in intracellular Ca\(^{2+}\), different stimuli are thought to achieve specificity by eliciting different spatiotemporal Ca\(^{2+}\) increases. We investigated the effect of nicotinic acid adenine dinucleotide phosphate (NAADP) inactivation on spatiotemporal Ca\(^{2+}\) signals in intact sea urchin eggs. The photorelease of NAADP but not inositol 1,4,5-trisphosphate or cyclic ADP-ribose resulted in self-inactivation. When NAADP was released first locally and subsequently globally, the spatial pattern of the first response shaped that of the second. Specifically, the local release of NAADP created a Ca\(^{2+}\) gradient that was reversed during the subsequent global release of NAADP. Neither cyclic ADP-ribose nor inositol 1,4,5-trisphosphate showed a similar effect. In contrast to homogenates, NAADP inactivation was reversible in intact eggs with resensitization occurring in ~20 min. Because initial NAADP responses affect later responses, NAADP can serve as a mechanism for a Ca\(^{2+}\) memory that has both spatial and temporal components. This NAADP-mediated Ca\(^{2+}\) memory provides a novel mechanism for cells to control spatiotemporal Ca\(^{2+}\) increases.

Numerous extracellular messengers control a diverse array of intracellular functions through increases in intracellular Ca\(^{2+}\) concentration (1). Nevertheless, intracellular targets can be selectively activated by Ca\(^{2+}\) through spatial and temporal control (1). One way of controlling spatiotemporal Ca\(^{2+}\) signaling is by modulating the sensitivity of intracellular Ca\(^{2+}\)-release channels to Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR). Through positive feedback, CICR results in regenerative Ca\(^{2+}\) release. Both inositol 1,4,5-trisphosphate (IP\(_3\)) and ryanodine receptors exhibit CICR, the sensitivity of which is controlled by IP\(_3\) (1) and cADPR (2), respectively. Thus, by altering the levels of these messengers, Ca\(^{2+}\) signals can be confined to subcellular regions or propagated throughout the cell (1).

The novel Ca\(^{2+}\)-mobilizing messenger nicotinic acid adenine dinucleotide phosphate (NAADP) was originally shown to mobilize Ca\(^{2+}\) in the sea urchin egg (3) and subsequently has been shown to be active in both mammalian (4–6) and plant tissues (7). Compared with IP\(_3\) and cADPR, NAADP has several unique characteristics in the sea urchin egg (2, 8). First, the response to a maximal NAADP concentration is eliminated by pretreatment with a subthreshold concentration of NAADP (9, 10). Second, NAADP binds to its receptor irreversibly (9, 11, 12), which may relate to the unique desensitization of NAADP (9) and increase the sensitivity of its receptor (12). Third, NAADP-mediated Ca\(^{2+}\) release is not regulated by Ca\(^{2+}\) (6, 13, 14). The unique properties of NAADP necessitate novel mechanisms for control of spatiotemporal Ca\(^{2+}\) signaling. For example, we recently reported that, in contrast to all previous Ca\(^{2+}\) waves that propagate by CICR (1), NAADP-mediated Ca\(^{2+}\) increases propagate by NAADP diffusion (15). We now show that NAADP desensitization-resensitization can establish a subcellular Ca\(^{2+}\) memory that has both spatial and temporal components. Thus, the unique properties of NAADP enables it to control spatiotemporal Ca\(^{2+}\) signaling in a fundamentally distinct manner from IP\(_3\) and cADPR.

**EXPERIMENTAL PROCEDURES**

Sea urchin eggs of *Lytechinus pictus* were obtained by intraocoelomic injection of 0.5 M KCl into artificial sea water (in mM, NaCl 435, MgCl\(_2\) 40, MgSO\(_4\) 15, CaCl\(_2\) 11, KCl 10, NaHCO\(_3\) 2.5, EDTA 1), dejellied by passing through 90-μm nylon mesh, and then washed twice by centrifugation. Eggs were transferred to polylysine-coated glass coverslips for microinjection and microscopy. Oregon Green 488 BAPTA (1,2-bis(2-aminophenoxy)ethane-N\(_2\)N\(_3\)N\(_3\)N\(_4\)-tetraacetic acid dextran; Molecular Probes) was pressure-microinjected (Picospritzer; World Precision Instruments). The Ca\(^{2+}\)-sensitive dye was imaged by laser-scanning confocal microscopy (Leica model TCS NT) using the 488-nm line of an argon ion laser for excitation, and the emission was long-pass filtered (515 nm) and detected with a photomultiplier tube. Caged NAADP (2'-P-(1-(2-nitrophenyl)ethyl) NAADP; Molecular Probes) was purified further by high performance liquid chromatography to remove small amounts of contaminating free NAADP (16). Caged cADPR (P-1,2-nitrophenoylethyl cADPR; Molecular Probes) and caged IP\(_3\) (4,5P-1,2-nitrophenoylethyl IP\(_3\); Calbiochem) were not further purified. Caged compounds were photolyzed with ultraviolet light (351- and 364-nm lines) from an argon ion laser (Enterprise model 651; Coherent) that was directed into the scanning head by a quartz fiber optic cable. The spatial location of photolysis was controlled via a shutter that was placed in the light path of the ultraviolet laser. This resulted in a band of UV across the image with the position and width of the band being controllable. The confocal images were processed with the software NIH Image to create a self ratio by dividing the intensity (F) of each image on a pixel by pixel basis by the intensity of an image acquired before stimulation (F\(_0\)).

**RESULTS AND DISCUSSION**

In sea urchin egg homogenates, Ca\(^{2+}\) release mediated by IP\(_3\), cADPR, and NAADP exhibit homologous desensitization,
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NAADP was photoreleased first locally and then, after Ca\(^{2+}\) had returned to near resting levels, globally. In the images the pseudocolor correspond to the Oregon Green 488 BAPTA dextran self ratio (F/F\(_0\)), and the images are taken from the times indicated by the vertical tick marks above the x axis of the graphs. The traces in the graph are coded by color to the regions of interest at the positions indicated in the diagram of the egg. The shading in the diagram of the egg indicates the area of photorelease, and the labeled horizontal bars above the traces and images indicate its duration. The response is representative of that observed in eight similar experiments.

but only the NAADP-sensitive Ca\(^{2+}\) release system inactivates to nonreleasing concentrations of itself (2, 3, 9, 10, 17, 18). The time course of NAADP self-inactivation is both time- and concentration-dependent (2, 9, 10). NAADP self-inactivation also occurs in intact eggs (2, 9, 10). To investigate the time course for messenger desensitization in intact eggs, each second messenger was photoreleased repeatedly at different time intervals (Fig. 1). In response to repeated stimulation with either IP\(_3\) or cADPR, the peak Ca\(^{2+}\) decreased, and the resting Ca\(^{2+}\) between pulses increased when the pulses were separated by 20 s but were largely unaffected when the pulses were separated by either 60- or 120-s intervals (Fig. 1). In contrast, in response to repeated stimulation with NAADP the peak Ca\(^{2+}\) decreased regardless of the time between the NAADP pulses (Fig. 1). The precise time course for NAADP inactivation in intact eggs cannot be determined accurately because of egg to egg variation and the fact that a given egg cannot be repeatedly stimulated with NAADP using different time courses.

A local release of NAADP resulted in a Ca\(^{2+}\) increase that spread only part way across an egg, thus forming a Ca\(^{2+}\) gradient (Fig. 2). We have demonstrated recently that NAADP-mediated Ca\(^{2+}\) increases spread via NAADP diffusion (15), rather than via Ca\(^{2+}\)-induced Ca\(^{2+}\) release, which underlies all previously described Ca\(^{2+}\) waves (1). When Ca\(^{2+}\) returned to near resting levels, a global release of NAADP resulted in a Ca\(^{2+}\) increase that was an inversion of the first Ca\(^{2+}\) gradient (Fig. 2). This second Ca\(^{2+}\) gradient is a direct consequence of the first NAADP gradient that formed subcellular zones where the NAADP response was completely inhibited, partially inhibited, and not inhibited.

In contrast to NAADP, a local release of either cADPR (Fig. 3a) or IP\(_3\) (Fig. 3b) did not affect the pattern of Ca\(^{2+}\) increase elicited by a later global release of messenger. These experiments illustrate a key difference between Ca\(^{2+}\) signals generated by IP\(_3\) and cADPR and those generated by NAADP. Namely, that NAADP responses exhibit a spatial memory of the previous response that shapes the spatial pattern of subsequent Ca\(^{2+}\) increases.

In sea urchin egg homogenates, NAADP inactivation lasts at least 25 min (9, 10) and correlates with irreversible binding of NAADP to its receptor (9). Similarly, in intact sea urchin eggs, when either a relatively large amount of NAADP was released (e.g., photoreleasing NAADP globally at a high UV intensity for several frames) or free NAADP was injected to a final intracellular concentration of 5 nM, the inactivation persisted for at least 2 h (data not shown). To determine the reversibility of spatially localized NAADP inactivation, eggs were locally desensitized to NAADP and then given various periods of recovery before a global release of NAADP. As shown in Fig. 2, when the local and global NAADP increases were separated by about 1 min, there was localization of inactivation (Fig. 4). As the recovery time between the two photoreleases of NAADP increased, the egg progressively recovered from inactivation until after about 23 min the NAADP-mediated Ca\(^{2+}\) increase was similar in both the regions (Fig. 4). To verify that the local region was desensitizing to NAADP, NAADP was released locally with a series of UV pulses. The amplitude of the response decreased with each additional UV pulse indicating desensitization. The subsequent response to global photorelease of NAADP (Fig. 4;
25 min) demonstrates that the recovery was genuine and not due to the lack of initial desensitization. Following the NAADP-mediated Ca\textsuperscript{2+} increase, certain eggs exhibited either a Ca\textsuperscript{2+} pulse (Fig. 4; 23 min, blue trace) or a small and sustained rise in Ca\textsuperscript{2+} (Fig. 4; 23 and 25 min). These secondary Ca\textsuperscript{2+} rises likely correspond to the Ca\textsuperscript{2+} oscillations induced by the photorelease of NAADP, as described previously by Aarhus et al. (9).

Although the mechanism for recovery of the NAADP response is currently unknown, the functional response (inhibition of NAADP-induced Ca\textsuperscript{2+} release) is relatively long-lasting in homogenates (9, 10) compared with intact eggs, suggesting that the sensitivity of the egg to NAADP is highly regulated in vivo. Taken together, the experiments shown in Fig. 4 demonstrate that NAADP inactivation is reversible when it is localized in intact eggs. Thus, the NAADP-mediated spatiotemporal Ca\textsuperscript{2+} memory is short term rather than permanent.

Our data demonstrate that in intact sea urchin eggs, NAADP inactivation can be spatially restricted and is reversible. These unique properties enable NAADP to form a spatiotemporal memory of past Ca\textsuperscript{2+} increases. Such a subcellular memory makes it ideal for controlling processes that require elevations in Ca\textsuperscript{2+} that are spatially restricted and maintain an influence for 10–20 min.

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