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Interactions between intracellular Ca^{2+} stores: Ca^{2+} released from the NAADP pool potentiates cADPR-induced Ca^{2+} release

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Abstract

Cells possess multiple intracellular Ca^{2+}-releasing systems. Sea urchin egg homogenates are a well-established model to study intracellular Ca^{2+} release. In the present study the mechanism of interaction between three intracellular Ca^{2+} pools, namely the nicotinic acid adenine dinucleotide phosphate (NAADP), the cyclic ADP-ribose (cADPR) and the inositol 1',4',5'-trisphosphate (IP_3)-regulated Ca^{2+} stores, is explored. The data indicate that the NAADP Ca^{2+} pool could be used to sensitize the cADPR system. In contrast, the IP_3 pool was not affected by the Ca^{2+} released by NAADP. The mechanism of potentiation of the cADPR-induced Ca^{2+} release, promoted by Ca^{2+} released from the NAADP pool, is mediated by the mechanism of Ca^{2+}-induced Ca^{2+} release. These data raise the possibility that the NAADP Ca^{2+} store may have a role as a regulator of the cellular sensitivity to cADPR.

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Introduction

The release of Ca^{2+} from intracellular stores is a widespread component of several signaling pathways (1-3). Nicotinic acid adenine dinucleotide phosphate (NAADP) is a recently discovered nucleotide with intracellular Ca^{2+}-releasing properties (4-11). NAADP-induced Ca^{2+} release was first described in sea urchin egg homogenates (5). The Ca^{2+} release mechanism elicited by NAADP differs in many ways from the Ca^{2+} release controlled by cyclic ADP-ribose (cADPR) and inositol 1',4',5'-trisphosphate (IP_3) (2,4-17). Properties of this Ca^{2+}-releasing molecule include: i) absence of regulation by the intracellular divalent cations Mg^{2+} and Ca^{2+} (6-8,15); ii) NAADP-induced Ca^{2+} release is fully inactivated by exposure to low concentrations of NAADP (14), and iii) Ca^{2+} release induced by NAADP appears to be insensitive to changes of pH over a wide range (8,17). These characteristics make NAADP a unique trigger of intracellular Ca^{2+} (2,9,10). In addition to the NAADP-induced Ca^{2+} release system, cells also possess other intracellular Ca^{2+} messengers such as cADPR and IP_3 (5). The exact physiological role of three different intracellular Ca^{2+}-releasing systems in cells is not known. However, it is possible that these different Ca^{2+} pools may interact in the complex mechanism of intra-

cellular Ca^{2+} oscillation (9,12,13,16,18). In the present study I explored *in vitro* the mechanisms by which NAADP could modulate the Ca^{2+} release elicited by cADPR. It was found that NAADP could potentiate the cADPR-induced Ca^{2+} release by sensitization of the ryanodine receptor by a mechanism similar to the Ca^{2+}-induced Ca^{2+} release. This result indicates that crosstalk between intracellular Ca^{2+} pools may modulate the complex mechanism of intracellular Ca^{2+} mobilization.

Material and Methods

Sea urchin egg homogenates

Homogenates from *Lytechinus pictus* egg were prepared as described previously (5). Frozen homogenates were thawed in a 17ºC water bath and diluted to 1.25% with an intracellular medium containing 250 mM N-methyl glutamine, 250 mM potassium gluconate, 20 mM HEPES buffer, pH 7.2, 1 mM $MgCl_2$, 2 U/ml creatine kinase, 4 mM phosphocreatine, 1 mM ATP, 3 µg/ml oligomycin, and 3 µg/ml antimycin. After incubation at 17ºC for 3 h, 3 µM fluo-3 was added. Fluo-3 fluorescence was monitored at 490 nm excitation and 535 nm emission in a 250-µl cuvette, held at 17ºC with a circulating water bath and continuously mixed with a magnetic stirring bar, in a Hitachi spectrofluorometer (F-2000).

^{45}Ca uptake and release were measured by a filtration method using glass-fiber filters as described in Ref. 6. The remaining intravesicular ^{45}Ca was determined by filtration of 0.2 ml of a 1.25% (v/v) egg homogenate through a prewashed GF/C glass filter (Whatman) under vacuum, followed by rapid washing three times with 1 ml of an ice-cold intracellular medium containing 3 mM $LaCl_3$. The radioactivity retained on the filter was determined by standard scintillation counting.

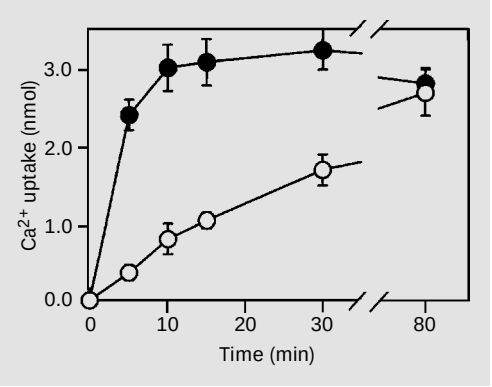

Figure 1. Ca^{2+} uptake in sea urchin egg homogenates. The determination of Ca^{2+} uptake was performed using ^{45}Ca as described in Material and Methods. Sea urchin egg homogenates were incubated in the presence (open circles) or absence (filled circles) of 10 µM thapsigargin (a Ca^{2+} ATPase inhibitor).

Material

L. pictus and *Aplysia californica* were obtained from Marinus Inc., Long Beach, CA, USA. Fluo-3 was purchased from Molecular Probes, Eugene, OR, USA, and IP_3, ryanodine, oligomycin and antimycin were from Calbiochem, San Diego, CA, USA. All other reagents, of the highest purity grade available, were supplied by Sigma Co., St. Louis, MO, USA. NAADP and cADPR were synthesized as described before (5).

The reported experiments were repeated at least three to six times.

Results and Discussion

NAADP and cADPR induce Ca^{2+} release from different Ca^{2+} pools

First we investigated the mechanisms of Ca^{2+} uptake in sea urchin egg homogenates, which were found to have both thapsigargin-sensitive and -insensitive Ca^{2+} uptake systems. These data indicate that egg homogenates have both a sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase (SERCA)-like pool and a second different mechanism of Ca^{2+} uptake that is not mediated by a SERCA-like enzyme. As shown in Figure 1, the thapsigargin-insensitive system is slower. However, the maximum amount of Ca^{2+} uptake was identical in the presence or absence of thapsigargin (Figure 1). Next we determined whether the intracellular Ca^{2+}-releasing

agents cADPR, IP_3, and NAADP could activate Ca^{2+} efflux in both thapsigargin-sensitive and -insensitive pools (Figure 2). In agreement with data previously reported by Genazzani and Galione (15), the results indicated that cADPR and IP_3 promoted Ca^{2+} release only through the thapsigargin-sensitive pools (Figure 2). In contrast, NAADP was able to induce Ca^{2+} release from both thapsigargin-sensitive and -insensitive pools (Figure 2), indicating that the NAADP and cADPR Ca^{2+} pools in sea urchin egg homogenates are at least partially independent.

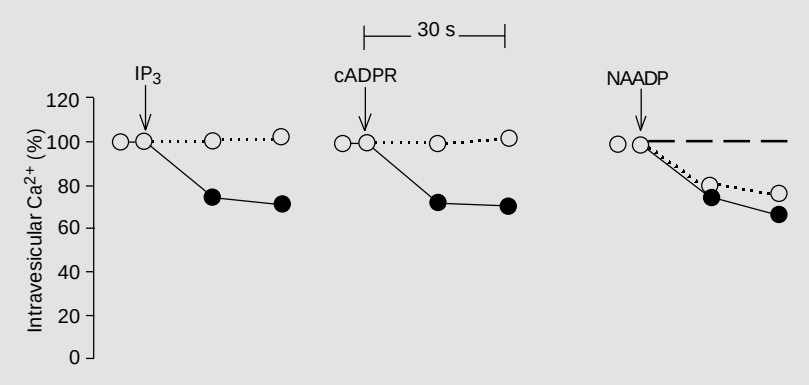

Figure 2. Ca^{2+} release induced by nicotinic acid adenine dinucleotide phosphate (NAADP) from the thapsigargin-insensitive pool. The sea urchin egg homogenates were loaded with ^{45}Ca as described in Figure 1. After 3 h of Ca^{2+} uptake, Ca^{2+} release was initiated by addition of 1 µM IP_3, 100 nM cyclic ADP-ribose (cADPR) or 100 nM NAADP. The Ca^{2+} release was performed in homogenates loaded in the absence (open circles) or the presence (filled circles) of 10 µM thapsigargin.

Potentiation of the Ca^{2+}-induced Ca^{2+} release by Ca^{2+} released from the NAADP pool

It has been previously reported that extravesicular Ca^{2+} can not only potentiate but is also necessary for the Ca^{2+} release induced by ryanodine receptor agonists such as cADPR and ryanodine (6,19). In contrast, the NAADP-induced Ca^{2+} release does not behave like a Ca^{2+}-induced Ca^{2+} release (6,15). It has been proposed that the Ca^{2+} released by NAADP could modulate the Ca^{2+}-induced Ca^{2+} release system activated by cADPR (18,20,21). However, no direct evidence for this action has been reported to date. Here we demonstrate that Ca^{2+} release from the NAADP pool could potentiate the Ca^{2+} release induced by ryanodine and cADPR. As shown in Figure 3, after the addition of 12 nM NAADP a small amount of Ca^{2+} was released from the vesicles, and the addition of subthreshold concentrations of cADPR at the peak (steady state) of the Ca^{2+} release led to a significant potentiation of the cADPR-induced Ca^{2+} release (Figure 3). This effect was not mediated by NAADP itself but by the increase in extravesicular Ca^{2+}, since when the Ca^{2+} release induced by NAADP was abolished by previous desensitization of the NAADP receptor the cADPR-induced Ca^{2+} release was not enhanced by NAADP (Figure 3C). The increase of extravesicular Ca^{2+} induced by NAADP in-

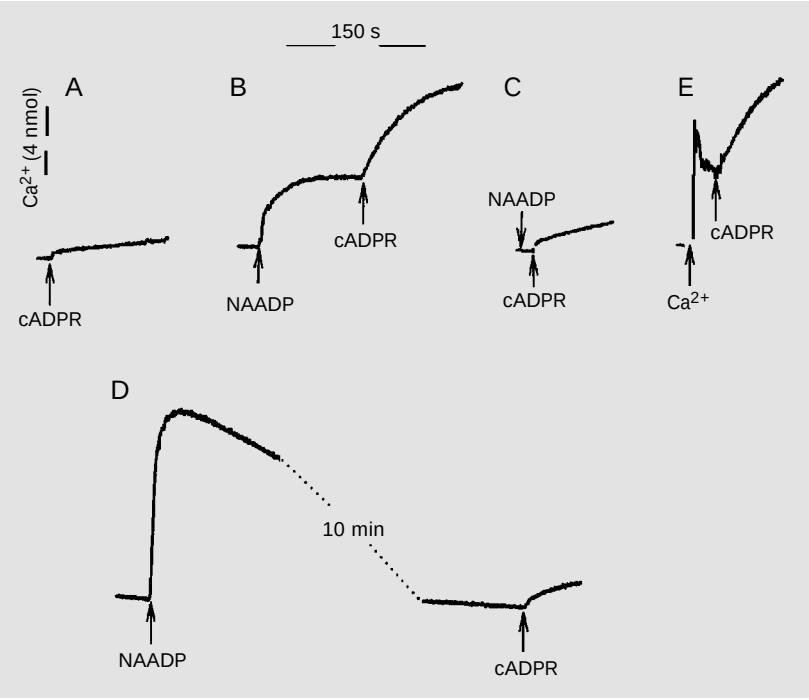

Figure 3. Potentiation of the cyclic ADP-ribose (cADPR)-induced Ca^{2+} release by Ca^{2+} released from the nicotinic acid adenine dinucleotide phosphate (NAADP) pool. Free Ca^{2+} concentrations were measured as described in Material and Methods using fluo-3. The arrow indicates the sequential addition of different Ca^{2+} channel agonists. In *A* the arrow indicates the addition of 16 nM cADPR that by itself does not promote Ca^{2+} release. In *B* the homogenate was first treated with 12 nM NAADP and 16 nM cADPR was added at the peak (steady state) of the Ca^{2+} release induced by NAADP. In *C* the homogenate was pretreated with 2 nM NAADP for 20 min (not shown) to promote self-desensitization of the NAADP receptor. After that the homogenate was treated with 12 nM NAADP and 16 nM cADPR. In *D* homogenates were treated with a saturating concentration of 60 nM NAADP and then, after the Ca^{2+} released by NAADP was taken up again, the homogenate was treated with 16 nM cADPR. In *E* the homogenate was treated with 4 nmol Ca^{2+} prior to the addition of 16 nM cADPR. The data are representative of 12 different experiments done with three different preparations of sea urchin egg homogenates.

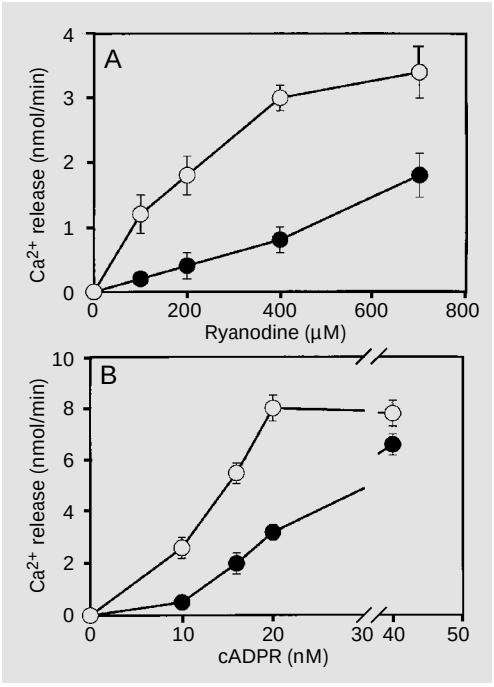

Figure 4. Effect of Ca^{2+} released by NAADP on the apparent affinity of the ryanodine receptor for ryanodine and cyclic ADP-ribose (cADPR). Homogenates were treated with no addition (filled circles), or with the addition of 12 nM NAADP (open circles) as shown in Figure 3B. The dose-response dependence for ryanodine (A) and cADPR (B) was determined by the addition of different concentrations of the Ca^{2+}-releasing compounds as shown in the figure. The addition of ryanodine and cADPR was performed after NAADP-induced Ca^{2+} release was at its plateau level (see Figure 3B). The Ca^{2+} released by NAADP potentiates the effect of both ryanodine and cADPR about 2.5 to 3 times. The data represent the mean ± SEM of four experiments.

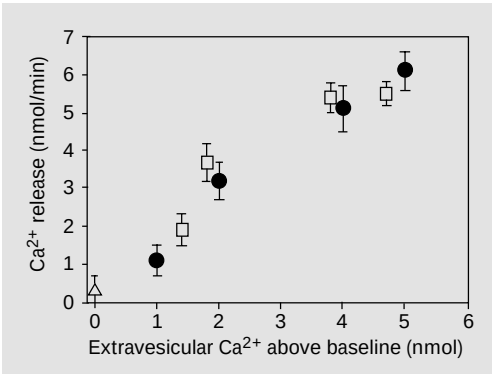

Figure 5. Effect of extravesicular Ca^{2+} on cADPR-induced Ca^{2+} release. Ca^{2+} release was monitored as described in Material and Methods. The figure indicates the Ca^{2+} released by 16 nM cADPR under different levels of extravesicular Ca^{2+} above baseline. The Ca^{2+} released under ambient extravesicular Ca^{2+} is indicated by a triangle. The extravesicular Ca^{2+} was increased by the addition of different concentrations of NAADP (squares) or Ca^{2+} (circles). The addition of cADPR was performed at the plateau level of Ca^{2+} induced by NAADP or Ca^{2+} itself, as shown in Figure 1. The data are the mean ± SEM of three independent experiments.

creased the apparent affinity of the ryanodine receptor for cADPR and ryanodine (Figure 4). Increasing the extravesicular Ca^{2+} could reproduce the effect of NAADP on the Ca^{2+} release mediated by cADPR by the addition of Ca^{2+} itself to the sea urchin egg homogenates (Figures 3E and 5). In fact, when normalized for the increase in extravesicular Ca^{2+} upon the potentiation of the cADPR-induced Ca^{2+} release, the effects of NAADP and of addition of Ca^{2+} itself were near identical (Figure 5). These data indicate that Ca^{2+} released from the NAADP pool can sensitize the ryanodine receptor to cADPR. In contrast, we found no effect of NAADP on the Ca^{2+} release induced by IP_3. Furthermore, Ca^{2+} released from the IP_3 pool was not consistently able to sensitize the cADPR-induced Ca^{2+} release (data not shown). This is probably due to the fact that cADPR and IP_3 induce Ca^{2+} release from the same Ca^{2+} pool in sea urchin egg homogenates (15).

A second mechanism for NAADP modulation of the cADPR-induced Ca^{2+} release has been described by Churchill and Galione (12), who reported that in intact sea urchin eggs NAADP-induced Ca^{2+} oscillations were mediated via a two-pool mechanism that primed the cADPR- and the IP_3-sensitive Ca^{2+} stores (12). In fact, priming the Ca^{2+} pools with Ca^{2+} (13) can increase the apparent affinity for cADPR and IP_3.

The precise role of NAADP-modulated Ca^{2+} release is not known. However, it has been proposed that in pancreatic acinar cells NAADP could be the trigger of Ca^{2+} oscillations induced by cholecystokinin (20,21). The cited investigators proposed that Ca^{2+} released by NAADP in response to cholecystokinin may activate the Ca^{2+}-induced Ca^{2+} release mediated by cADPR, leading to amplification of the Ca^{2+} signaling and generation of the Ca^{2+} oscillation (20,21). A similar role for NAADP has been proposed for the mobilization of Ca^{2+} in starfish oocytes (18). The present study is the first to demonstrate a direct effect of the Ca^{2+} released by NAADP on the apparent affinity of the ryanodine receptor for cADPR (Figure 4). This further indicates that NAADP may have an important role in the complex mechanism of intracellular Ca^{2+} mobilization in several vertebrate and invertebrate cells (4,5,16-18,20,21). In fact, the Ca^{2+} released from the NAADP pool can modulate the intracellular Ca^{2+} release by at least two different mechanisms: a) by priming the intracellular Ca^{2+} pools (16) and b) by direct sensitization of the Ca^{2+}-induced Ca^{2+} release.

Multiple intracellular Ca^{2+} stores are present in many cells (1,4-6,20,21) and may play a role in several physiological processes including muscle contraction, exocrine and endocrine secretion, fertilization, neuronal activation and immune cell function (1,2,9, 13,16-18,20). Exactly how Ca^{2+} exerts its intracellular effects is not completely understood. The answer may lie in the complex interaction between intracellular and extracellular Ca^{2+} pools to generate specific spatial-temporal intracellular Ca^{2+} signals. In this regard, the present results describing the direct interactions between NAADP (a non-Ca^{2+}-induced Ca^{2+} release) and cADPR (a Ca^{2+}-induced Ca^{2+} release) Ca^{2+} stores may be of broad physiological importance. In fact, the determination of the specific role of different Ca^{2+} stores in several cellular functions deserves further investigation.

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