Nicotinamide inhibits cyclic ADP-ribose-mediated calcium signalling in sea urchin eggs

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Cyclic ADP ribose (cADPR) is a potent Ca^{2+} -releasing agent, and putative second messenger, the endogenous levels of which are tightly regulated by synthetic (ADP-ribosyl cyclases) and degradative (cADPR hydrolase) enzymes. These enzymes have been characterized in a number of mammalian and invertebrate tissues and their activities are often found on a single polypeptide. β -NAD⁺, cGMP and nitric oxide (NO) have been reported to mobilize Ca^{2+} in the sea urchin egg via the cADPR-mediated pathway. We now report that in sea urchin egg homogenates, nicotinamide inhibits the Ca^{2+} -mobilizing action of β -NAD⁺, cGMP and NO, but has no effect on cADPR-induced Ca^{2+} release. Moreover, nicotinamide inhibits cGMP-induced rege-

nerative Ca^{2+} waves in the intact sea urchin egg. By successfully separating the cADPR-metabolizing machinery from that which releases Ca^{2+} , we have shown that nicotinamide inhibits cADPR-mediated Ca^{2+} signalling at the level of cADPR generation. Importantly, nicotinamide had no effect upon the hydrolysis of cADPR, and its selective action on cyclase activity was supported by its inhibition of purified *Aplysia* ADP-ribosyl cyclase, which does not exhibit detectable hydrolytic activity. The action of nicotinamide in blocking Ca^{2+} release by β -NAD⁺, cGMP and NO strongly suggests that these agents act as modulators of cADPR synthesis rather than to sensitize calcium release channels to cADPR.

INTRODUCTION

NAD glycohydrolases (NADases) are a class of enzymes that catalyse the hydrolysis of oxidized nicotinamide adenine dinucleotide (β -NAD⁺) to adenosine diphosphate ribose (ADPR) and nicotinamide [1–3]. These enzymes are ubiquitously distributed, both intra- and extracellularly, and have been purified from eukaryotic and prokaryotic sources [4]. Important roles in cellular regulation have been suggested for NADases and the related ADP-ribosyl transferases. The poly(ADP-ribosyl) transferases are predominantly nuclear enzymes involved in the synthesis of poly(ADP-ribose) in response to DNA damage or cellular division, whereas intrinsic (and toxin-induced) mono-ADP-ribosylation occurs on proteins in the plasma membrane and may be involved in the regulation of signalling pathways or membrane traffic [5,6].

The recently discovered ADP-ribosyl cyclases resemble NADases as they also catalyse the overall hydrolysis of β -NAD⁺ to ADPR and nicotinamide. However, their distinguishing function is that of cyclizing the ribose-nucleotide, following cleavage of the nicotinamide-ribose linkage, to form cyclic ADP-ribose (cADPR). To date, four ADP-ribosyl cyclases have been purified, namely; CD38 (a lymphocyte differentiation antigen), BST-1 (a bone-marrow stromal cell-surface molecule), canine spleen NADase, and *Streptococcus pyogenes* NADase. All exhibit synthetic (ADP-ribosyl cyclase) and degradative (cADPR hydrolase) activities on the same polypeptide and are thus bifunctional enzymes [7–11]. A notable exception is *Aplysia* ADP-ribosyl cyclase, which exhibits little detectable hydrolase activity [12]. The similarities between ADP-ribosyl cyclases and NADases has led to the hypothesis that classical NADases are also bifunctional but do not release their intermediate cADPR [3].

Unlike nicotinamide and ADPR, the cyclic product, cADPR, is a potent Ca^{2+} -mobilizing agent. It was this action, in sea urchin egg homogenates, that led to the original discovery of cADPR and its metabolizing enzymes [12,13]. cADPR is now known to

be a widespread activator of Ca^{2+} -induced Ca^{2+} release (CICR) by ryanodine-sensitive (InsP_3 -insensitive) Ca^{2+} channels in several invertebrate and mammalian cell types [14–16]. It may also function as a second messenger involved in the regenerative Ca^{2+} waves seen in sea urchin eggs at fertilization [17,18].

If cADPR is an endogenous modulator of CICR and/or a second messenger involved in response to primary signals at the cell surface, there must exist mechanisms for regulating its intracellular levels. To date the only handle available on such mechanisms is the proposed involvement of a cGMP-dependent stimulation of ADP-ribosyl cyclase activity. This suggests cADPR synthesis may be enhanced following elevation of cGMP levels via nitric oxide (NO) or cell-surface receptors, which activate guanylate cyclases [19–21]. Recently, cGMP-induced Ca^{2+} release, via a similar InsP_3 -independent pathway, has also been demonstrated in mammalian Jurkat [22] and dorsal root ganglion [23] cells. Whether the recently described stimulation of ADP-ribosyl cyclases in longitudinal smooth muscle by cholecystokinin [24] involves either NO or cGMP as intermediates, remains to be determined.

In this study we report the observation that nicotinamide inhibits Ca^{2+} release by these agents in sea urchin eggs and demonstrate that these actions occur through an inhibition of ADP-ribosyl cyclase activity.

MATERIALS AND METHODS

Preparation of sea urchin egg homogenates

Eggs were obtained by stimulating ovulation of *Lytechinus pictus* (Marinus, Inc., Long Beach, CA, U.S.A.) with an intracoelomic injection (up to 0.5 ml) of 0.5 M KCl solution. They were collected in artificial sea water (ASW; 435 mM NaCl, 40 mM MgCl_2 , 15 mM MgSO_4 , 11 mM CaCl_2 , 10 mM KCl, 2.5 mM NaHCO_3 , 1 mM EDTA, pH 8.0), and jelly was removed by filtration through 85 μm Nitex mesh. Eggs were washed (in

Abbreviations used: NADase, NAD glycohydrolase; cADPR, cyclic ADP-ribose; ADPR, ADP-ribose; NO, nitric oxide; CICR, Ca^{2+} -induced Ca^{2+} release; ASW, artificial sea water; Glu-IM, intracellular medium.

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approx. 10 times egg volume, at room temperature) twice in Ca^{2+} -free ASW (comprising 470 mM NaCl, 27 mM MgCl_2 , 28 mM MgSO_4 , 10 mM KCl, 2.5 mM NaHCO_3 and 1 mM EGTA, pH 8.0), twice in Ca^{2+} -free ASW without EGTA and once in Intracellular medium (Glu-IM) (comprising: 250 mM *N*-methylglucamine, 250 mM potassium gluconate, 20 mM Hepes and 1 mM MgCl_2 , pH titrated to 7.2 with acetic acid). Eggs were then resuspended to 50% (v/v) in homogenization buffer (Glu-IM containing 1 mM ATP, 10 units/ml creatine phosphokinase, 10 mM phosphocreatine, 25 $\mu\text{g/ml}$ leupeptin, 20 $\mu\text{g/ml}$ aprotinin and 100 $\mu\text{g/ml}$ soybean trypsin inhibitor). Eggs were homogenized at 4 °C in a Dounce homogenizer using a type 'B' pestle (six strokes) and immediately centrifuged at 13000 *g* for 8 s. The supernatant (50% homogenate) was collected and stored at -70 °C (in 500 μl aliquots) until use.

Preparation of purified microsomes and supernatant fractions

Microsomes were purified from 50% egg homogenates by the Percoll density centrifugation method described previously [13]. Briefly, a fractionating buffer was prepared by diluting Percoll stock to 25% in Ac-IM stock (consisting of 333 mM *N*-methylglucamine, 333 mM potassium acetate, 27 mM Hepes, 1.3 mM MgCl_2 , pH titrated to 7.2 with acetic acid). This was then supplemented with 0.5 mM ATP, 2 units/ml creatine phosphokinase, 4 mM phosphocreatine and 10 μM EGTA. A 1 ml sample of 50% egg homogenate was layered on 6 ml of this solution and centrifuged at 27000 *g* for 30 min at 15 °C. The top 1 ml fraction was collected and represented the 50% supernatant fraction. This contained ADP-ribosyl cyclase activity. The Ca^{2+} -storing, cADPR-sensitive microsomes formed a distinct tight band halfway down the tube. A 1 ml aliquot of this was collected (50% microsomes) using a disposable syringe to puncture the vessel wall. The fractions were collected and stored at -70 °C (in 500 μl aliquots) until use.

ADP-ribosyl cyclase activity (discontinuous assay)

Catalytic activities of *L. pictus* cyclase and purified *Aplysia* cyclase were measured using a discontinuous assay whereby 5 μl of reaction mixture was withdrawn and assayed immediately for cADPR content in the bioassay described below. The reactions were conducted in Glu-IM (for sea urchin egg extract) or 20 mM Tris (pH 7.0) (for purified *Aplysia* cyclase) with various concentrations of $\beta\text{-NAD}^+$ and nicotinamide. Samples of 25% egg extract (approx. 5.2 mg of protein/ml) or 1–3 m-units of *Aplysia* cyclase were added to each mix to a final volume of 50 μl . Reaction mixtures were adjusted to 17 °C and reactions were initiated by the addition of the enzyme. Incubations lasted for 90 min (egg extract) or 5 min (*Aplysia* cyclase) at 17 °C.

cADPR hydrolase activity was assessed by following the disappearance of 2 μM cADPR with or without nicotinamide (20 mM) under the same conditions.

Fluorimetry and estimation of cADPR (bioassay)

cADPR was assayed using a routine calcium release bioassay in 2.5% sea urchin (*L. pictus*) homogenates as described previously [25]. Briefly 50% egg homogenates were thawed at room temperature and sequentially diluted (three times) to 2.5 or 5% in homogenization buffer supplemented with 1 $\mu\text{g/ml}$ oligomycin, 1 $\mu\text{g/ml}$ antimycin, 1 mM sodium azide and 3 μM fluo-3. Each dilution step involved a 60 min incubation at 17 °C.

Fluorimetry was performed at 17 °C using 500 μl of 2.5% homogenate, continuously stirred, in a Perkin-Elmer LS-50B fluorimeter. Free Ca^{2+} concentration was measured by moni-

toring fluorescence intensity at excitation and emission wavelengths of 490 nm and 535 nm, respectively. A cADPR concentration–response curve was constructed using standards added in Glu-IM containing 10 μM EGTA. When necessary this was adapted (sensitized by a 1 min pre-incubation with 2 mM caffeine) to allow estimation of lower levels of cADPR (0.2–5 μM) instead of 2–20 μM in the absence of caffeine [26]. All additions were made in 5 μl volumes.

Imaging of intracellular free Ca^{2+} in intact eggs

Dejellied eggs were transferred to poly(L-lysine) (10 mg/ml)-coated glass coverslips, allowed to adhere and micro-injected with fura-2, pentapotassium salt (2 mM in the pipette), in buffer consisting of 0.5 M KCl/20 mM Pipes, pH 6.7, to a final cellular concentration of approx. 2 μM . Injection volumes were approx. 1% of cell volume. All experiments were performed at 22 °C. Free cytosolic Ca^{2+} concentration was determined by comparing the ratios of fluorescence intensities at excitation wavelengths of 340 nm and 380 nm, using an emission wavelength of 510 nm. Ratio images were obtained using a fluorimetric imaging system and 'Ionvision' software supplied by Improvision Ltd, University of Warwick Science Park, Coventry, U.K. Standard CaCl_2 solutions were used to calibrate the system and viscosity corrections were made [27].

Materials

Fluo-3 and fura-2 were purchased from Molecular Probes. All other chemicals, including ADP-ribosyl cyclase from *Aplysia californica*, were from Sigma.

RESULTS

Effect of nicotinamide on $\beta\text{-NAD}^+$ -induced Ca^{2+} release

Figure 1(A) shows the Ca^{2+} -mobilizing effect of 150 μM $\beta\text{-NAD}^+$

Figure 1 Effect of nicotinamide on $\beta\text{-NAD}^+$ - and cADPR-induced Ca^{2+} mobilization

A sample of 5% sea urchin egg homogenate containing the Ca^{2+} -sensitive fluorescent probe, fluo-3, was incubated with 5 mM nicotinamide (NiAm) 2 min prior to control treatments of (A) $\beta\text{-NAD}^+$ (150 μM) and (B) cADPR (100 nM).

Figure 2 Effect of nicotinamide on cADPR production by sea urchin egg extract

A 25% sea urchin egg supernatant extract was incubated with 2 mM β -NAD⁺ and nicotinamide (NiAm) (0–4 mM) at 17 °C in Glu-IM buffer. cADPR levels in 5 μ l samples were assayed immediately at 90 min following the initiation of the reactions. (A) Representative fluorimetric traces obtained during the bioassay in 2.5% sea urchin egg homogenate. Where indicated, 8NH₂-cADPR was added to the bioassay homogenate prior to addition of control sample. (B) Fluorimetric traces used to calibrate the bioassay (5 μ l additions of cADPR standards: 3.9–62.5 pmol). (C) Concentration-dependent inhibition of cADPR production by nicotinamide. Each value represents a mean of triplicate determinations $\pm$ S.D.

on 5% sea urchin egg homogenates. Unlike the immediate cADPR-induced Ca²⁺ release (Figure 1B), the β -NAD⁺-induced Ca²⁺ release was preceded by a latent period, indicative of its enzymic conversion into cADPR [13]. Addition of nicotinamide (5 mM), 2 min before the application of β -NAD⁺, completely abolished the β -NAD⁺-induced Ca²⁺ mobilization (Figure 1A). This concentration of nicotinamide had no effect on submaximal cADPR-induced Ca²⁺ release (Figure 1B) and so was unlikely to be due to a direct effect on the cADPR-sensitive Ca²⁺ release channel.

Effect of nicotinamide on ADP-ribosyl cyclase activity

To investigate the action of nicotinamide on the synthesis of cADPR, crude sea urchin egg extracts (containing ADP-ribosyl cyclase) were incubated with 2 mM β -NAD⁺ and increasing concentrations of nicotinamide. cADPR production was assayed using the Ca²⁺-release bioassay in 2.5% sea urchin egg homogenate (see the Materials and methods section and also Figures 2A and 2B). We confirmed that the Ca²⁺-mobilizing agent in the assay samples was cADPR, by verifying with cross-desensitization experiments using authentic cADPR (results not shown) and by inhibiting sample-induced Ca²⁺ with a pre-addition of the cADPR antagonist 8NH₂-cADPR [26] to the assay homogenate (Figure 2A). The amount of cADPR produced by 2 mM β -NAD⁺, in the absence of nicotinamide, by 25% sea urchin egg

Figure 3 (A) Inhibition of cADPR synthesis by purified *Aplysia* cyclase and (B) effect of isonicotinic acid hydrazide on cADPR production by sea urchin egg extract

(A) *Aplysia* cyclase (20 ng/ml) was incubated with β -NAD⁺ (500 μ M) and 0–4 mM nicotinamide ($\blacktriangle$), or 0–5 mM isonicotinic acid hydrazide ($\circ$) in 20 mM Tris, pH 7.0. Cyclic ADPR levels were estimated immediately after 5 min incubations at 17 °C. Each value represents the mean of triplicates $\pm$ S.D. Control incubations produced 2.4 ± 0.5 nmol of cADPR/ng of protein per 5 min. (B) A sample of 25% sea urchin egg supernatant extract was incubated with 2 mM β -NAD⁺ and 0–5 mM isonicotinic acid hydrazide at 17 °C in Glu-IM buffer. cADPR levels in 5 μ l samples were assayed at 90 min following the initiation of the reactions. Each value represents a mean of triplicate determinations $\pm$ S.D.

extract was 0.558 ± 0.04 nmol of cADPR/mg of protein per 90 min ($n = 6$). Incubations of 25% sea urchin egg extract without β -NAD⁺ did not evoke any Ca²⁺ transients in the bioassay (results not shown). Figures 2(A) and 2(C) show that nicotinamide inhibited the production of cADPR by sea urchin extracts, in a concentration-dependent manner, with an IC₅₀ of approx. 1.5 mM (Figure 2C).

As sea urchin extracts also exhibit appreciable cADPR-hydrolase activity [28], nicotinamide could be decreasing cADPR levels by stimulating the degradation of cADPR. This was tested by monitoring the disappearance of cADPR in sea urchin extracts in the presence and absence of nicotinamide. The hydrolysis of cADPR (2 μ M) by sea urchin cADPR hydrolase ($t_{1/2} = 50 \pm 2.5$ min; $n = 3$) was unaffected by the presence of high concentrations of nicotinamide (20 mM) ($t_{1/2} = 49 \pm 2.8$ min; $n = 3$). Therefore, nicotinamide did not stimulate cADPR hydrolysis under the same conditions.

That nicotinamide reduces cADPR levels by inhibiting cyclase activity, and not by stimulating hydrolysis, is further supported by the finding that nicotinamide inhibited cADPR production by purified *Aplysia* cyclase, an enzyme that lacks detectable cADPR hydrolase activity. Figure 3(A) (triangles) shows that nicotinamide inhibited cADPR synthesis by *Aplysia* cyclase, in a concentration-dependent manner, with an IC₅₀ for nicotinamide of approx. 0.04 mM.

Figure 4 Effect of nicotinamide on NO- and cGMP-induced Ca^{2+} release in sea urchin egg homogenates

Nicotinamide (NiAm; 5 mM) was added to 5% sea urchin egg homogenate 2 min prior to control treatment. **(A)** Effect of nicotinamide on cGMP-induced Ca^{2+} release. $\beta\text{-NAD}^+$ (75 μM) was added 40 s prior to cGMP (75 μM). **(B)** Effect of nicotinamide on NO-induced Ca^{2+} release. GTP (250 μM) and $\beta\text{-NAD}^+$ (75 μM) were added at 60 s and 40 s (respectively) prior to NO (10.5 μM). Application of NO was made as previously reported [21]. The control Ca^{2+} transients were similar to those induced by larger concentrations of the cyclase substrate alone (150 μM) (Figure 1A), and are preceded by a latency of 160 and 300 s for NO and cGMP respectively.

Effect of NADase inhibitor, isonicotinic acid hydrazide, on cADPR synthesis

To distinguish mono-ADP-ribosylation from ADP-ribosyl cyclase activity, the action of isonicotinic acid hydrazide, a selective inhibitor of NADase, was tested on cADPR production by *Aplysia* cyclase (Figure 3A, circles) and sea urchin egg extracts (Figure 3B). Like nicotinamide, isonicotinic acid hydrazide inhibited cADPR synthesis by both enzymes, in a concentration-dependent manner. However, isonicotinic acid hydrazide was found to be less potent ($\text{IC}_{50} = 1.6$ mM for *Aplysia* cyclase and $\text{IC}_{50} = 90$ mM for *L. pictus* egg extracts).

Effect of nicotinamide on signalling pathways involving cADPR-induced Ca^{2+} mobilization

An indication that cADPR synthesis is enhanced by cGMP and NO is the absolute dependence on $\beta\text{-NAD}^+$ and significant decrease in this substrate upon the addition of cGMP [19]. As an inhibitor of ADP-ribosyl cyclases, nicotinamide should block signal transduction pathways involving cADPR synthesis. Indeed, in 5% sea urchin egg homogenate, the NO- and cGMP-induced Ca^{2+} responses were completely abolished by the cyclase inhibitor, nicotinamide (Figure 4). This supports the proposed mechanisms of NO- and cGMP-induced Ca^{2+} release which act by increasing cADPR levels and release Ca^{2+} from cADPR-sensitive stores.

That a similar modulatory mechanism exists in the intact egg is demonstrated in Figure 5. cGMP injections into fura-2-loaded cells induced Ca^{2+} release that propagated across the eggs as a regenerative Ca^{2+} wave. The peak Ca^{2+} response occurred approx. 70 ± 32.5 s ($n = 6$) after the initial injection and had a mean amplitude of 1214 ± 206 nM ($n = 6$). In the presence of 20 mM

Figure 5 Inhibition of cGMP-induced Ca^{2+} waves in intact eggs

Digital ratio images measured at 22 °C, with the Ca^{2+} -sensitive dye fura 2, are displayed at the times indicated and are representative of at least six separate experiments. Arrows indicate cGMP injections giving a final concentration of 250 μM (25 mM in the pipette). **(A)** Control cGMP injections induced a calcium transient (1214 ± 206 nM; $n = 6$) which took 70 ± 32.5 s to peak. The Ca^{2+} transients always returned to baseline within 8–10 min after cGMP injections. **(B)** Eggs pretreated with bathing solution containing nicotinamide (20 mM) produced a significantly smaller Ca^{2+} signal (200 ± 57 nM; $n = 4$ and $P = 1.56 \times 10^{-5}$). The time taken to peak Ca^{2+} appeared unchanged, mean = 81 ± 28 s ($P = 0.77$).

nicotinamide (added to the bathing solution), the Ca^{2+} transients were reduced to 200 ± 57 nM ($n = 4$, $P = 3 \times 10^{-5}$). The time taken for Ca^{2+} levels to peak from the moment of injection was unchanged in the presence of nicotinamide (mean = 81 ± 28 s; $P = 0.77$).

DISCUSSION

We report here that in sea urchin eggs nicotinamide inhibits $\beta\text{-NAD}^+$ -, cGMP-, and NO-induced Ca^{2+} release *in vitro* and *in vivo*. It does this by inhibiting ADP-ribosyl cyclase activity and hence cADPR synthesis. These actions of nicotinamide are consistent with previous reports showing inhibition of two other purified cyclases (*Aplysia kurodai* cyclase and CD38) [29,30].

NADases, which bear some similarities to ADP-ribosyl cyclases, are also inhibited by nicotinamide through both competitive [31] and non-competitive mechanisms [32]. However, the mechanism by which nicotinamide inhibits the ADP-ribosyl cyclases is different and has been described as mixed or complex inhibition and may be due to the reversibility of the forward reaction [29,30]. Under the incubation conditions used in the present study the reverse reaction was most unlikely, as the presence of nicotinamide did not enhance the disappearance of cADPR, suggesting that hydrolysis of cADPR was also unaffected.

We were unable to continuously assay for cyclase activity in egg extracts, by monitoring the production of fluorescent cyclic product, cGDP-ribose, from the non-fluorescent substrate, nicotinamide guanine dinucleotide (NGD⁺) [33]. This is perhaps due to a combination of low enzyme activity and quenching of the fluorescence by the high absorbance of the preparation. Clearly, purification of the sea urchin enzyme system is required to address these issues further. That nicotinamide did not inhibit cADPR-induced Ca^{2+} release (Figure 1) means that nicotinamide-treated egg homogenates can now be used in an adapted cADPR

bioassay. This will be particularly useful for assaying crude tissue extracts containing much higher levels of β -NAD⁺. Thus any contribution of β -NAD⁺ to the Ca²⁺ release in the bioassay is eliminated and the detection of ADP-ribosyl cyclase activity is enhanced.

Previous studies have shown that nicotinamide has a number of other effects and we must consider these during the interpretation of our results. Nicotinamide, as a product of β -NAD⁺ hydrolysis, will inhibit all β -NAD⁺-metabolizing enzymes including ADP-ribosyl transferases that have been implicated in other signal transduction pathways. Poly(ADP-ribosyl) synthetases are located in the nucleus, where they are important for DNA synthesis and repair [5,6], but these are unlikely to participate in the cytosolic Ca²⁺-mobilizing pathways in our system. However, mono-(ADP-ribosyl) transferases, which ADP-ribosylate membrane proteins, have been implicated in two other Ca²⁺-mobilizing signal transduction pathways. These are (1) oxidant-induced Ca²⁺ release from mitochondria [34,35], and (2) T-cell-receptor-mediated (TCR-mediated) transmembrane signalling in cytotoxic T lymphocytes [36], both of which were blocked by nicotinamide (20 mM). We think it unlikely that nicotinamide is exerting its effects via inhibition of mono-ADP-ribosylation in our system since the weak NADase inhibitor, isonicotinic acid hydrazide, which does not inhibit mono-ADP-ribosylation [37,38], had similar inhibitory effects to nicotinamide (Figures 3 and 4).

Previous studies in the sea urchin egg system have suggested that cADPR may mediate intracellular actions of cGMP on Ca²⁺ release. In the whole egg we showed that nicotinamide blocked the cGMP-induced Ca²⁺ wave (Figure 5) that presumably arises from an increase in cADPR levels within the egg. Together with the *in vitro* data this result strongly suggests that ADP-ribosyl cyclase activity is required for the initiation and propagation of the cGMP-induced Ca²⁺ wave.

Nicotinamide has also been used to increase cADPR levels by elevating endogenous β -NAD⁺ [39–41]. These results seem to conflict with our report but can be explained by the fact that both previous studies used pre-incubations of nicotinamide (2–5 mM), which is then diluted out during the proposed agonist-induced stimulation of cADPR synthesis. In contrast nicotinamide is present throughout all our measurements of ADP-ribosyl cyclase activity.

In summary we have shown that in sea urchin eggs, nicotinamide acts on ADP-ribosyl cyclase to inhibit cGMP-induced Ca²⁺ release *in vitro* and *in vivo*. This supports the idea that cGMP (and also NO) exert their actions by stimulating cADPR synthesis.

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