Nicotinic acid–adenine dinucleotide phosphate (NAADP) elicits specific microsomal Ca^{2+} release from mammalian cells

Ahad N.-K. YUSUFI, Jingfei CHENG, Michael A. THOMPSON, Eduardo N. CHINI and Joseph P. GRANDE[1]

Renal Pathophysiology Laboratory, Department of Physiology and Biophysics, Division of Nephrology, Department of Medicine, Mayo Clinic, Mayo Medical School, Rochester, MN 55905, U.S.A.

Nicotinic acid–adenine dinucleotide phosphate (NAADP), a molecule derived from β-NADP, has been shown to promote intracellular calcium release in sea urchin eggs. However, there is little information regarding the role of NAADP in the regulation of intracellular calcium fluxes in mammalian cells. We found recently that several mammalian tissues have a high capacity for NAADP synthesis, as assessed by sea urchin egg bioassay. To determine the functional significance of NAADP production by mammalian tissues, we sought to determine whether NAADP is capable of inducing calcium release from microsomes prepared from cultured cells. We found that NAADP, but not β-NADP, activates a specific microsomal calcium release system in mesangial cells isolated from rat kidney; NAADP was without effect in renal tubular epithelial cells. NAADP-induced calcium release is not affected by inhibitors of the inositol 1,4,5-trisphosphate or ryanodine channels. However, NAADP-elicited calcium release was inhibited by L-type calcium channel blockers and by alkaline phosphatase treatment of NAADP. NAADP also promotes specific microsomal calcium release in rat vascular smooth muscle cells, cardiac myocytes, fibroblasts and a human leukaemia cell line, indicating that the capacity for NAADP-induced calcium release is widespread in mammalian cells. We propose that NAADP may be an important regulator of intracellular calcium in many mammalian tissues.

Key words: cyclic ADP-ribose, calcium, inositol trisphosphate, mesangial cells, ryanodine.

INTRODUCTION

Calcium is a well recognized intracellular second messenger that regulates a wide variety of cellular homoeostatic functions and adaptive responses to injury, including contractility, protein synthesis and turnover, hormone secretion, proliferation and activation [1–3]. Several functionally distinct signalling pathways that regulate intracellular calcium release have been identified. Among these, the most completely characterized and most widespread is the inositol 1,4,5-trisphosphate (IP_3) system, which is a rapid-onset pathway triggered by the binding of vasoactive peptides or other short-acting hormones to a receptor coupled to phosphatidylinositol-specific phospholipase C, which catalyses the conversion of phosphatidylinositol 4,5-bisphosphate into IP_3 [4,5]. The IP_3 generated by this system binds a specific IP_3 receptor/channel, leading to the cytoplasmic release of calcium from intracellular stores [6]. A second major calcium signalling pathway is mediated by cyclic ADP-ribose (cADPR), which is synthesized by the enzyme ADP-ribosyl cyclase [7,8]. cADPR increases the sensitivity of the ryanodine receptor/channel to calcium, thereby stimulating calcium-induced calcium release [9,10].

Studies of intracellular calcium release in sea urchin eggs have led to the identification of a distinct, novel calcium signalling pathway activated by nicotinic acid–adenine dinucleotide phosphate (NAADP), an analogue of β-NADP also produced by ADP-ribosyl cyclase [11–14]. NAADP, in nanomolar concentrations, triggers calcium release via a mechanism that is fundamentally different from those controlled by IP_3 or cADPR [12]. The relevance of NAADP to calcium signalling in mammalian cells remains to be clearly established. Employing a sea urchin egg bioassay, we demonstrated that many rat tissues, including brain, heart, liver and spleen, have a capacity for NAADP synthesis [15]. However, to date, NAADP-elicited calcium release in mammalian cells has been documented only in pancreatic acinar cells and brain microsomes [16,17]. In the present studies, we sought to determine whether the capacity for NAADP-mediated calcium release is widespread in mammalian cells. NAADP induces calcium release from rat mesangial cell microsomes in a dose-dependent fashion, through a pathway that is distinct from those involving cADPR or IP_3. Conversely, NAADP does not promote the release of calcium from renal tubular epithelial cells, which have little capacity for NAADP synthesis, as assessed by sea urchin egg bioassay [15,18]. NAADP also stimulates calcium release in microsomes isolated from vascular smooth muscle cells, cardiac myocytes, fibroblasts and HL-60 cells (a human leukaemia cell line), indicating that the capacity for NAADP-induced calcium release is widespread in mammalian cells.

MATERIALS AND METHODS

Materials

[^{3}H]Ryanodine and $^{45}Ca^{2+}$ were purchased from Amersham (Arlington, IL, U.S.A.). cADPR, 8-bromo-cADPR, ADP-ribose and Ruthenium Red were purchased from Calbiochem (La Jolla, CA, U.S.A.). Heparin and β-NADP were purchased from Sigma (St. Louis, MO, U.S.A.). Tissue culture reagents were purchased from Gibco BRL (Gaithersburg, MD, U.S.A.). *Lytechinus pictus* was obtained from Marinus Corp. (Long Beach, CA, U.S.A.). Fluo-3 was purchased from Molecular Probes (Eugene, OR, U.S.A.).

Cell culture

Glomeruli were isolated from male Sprague–Dawley rats (body weight $\sim$ 200 g) by differential sieving, as previously described [19–21]. Cell outgrowths were characterized as mesangial cells by

Abbreviations used: cADPR, cyclic ADP-ribose; IP_3, inositol 1,4,5-trisphosphate; NAADP, nicotinic acid–adenine dinucleotide phosphate.

[1] To whom correspondence should be addressed (e-mail grande.joseph@mayo.edu).

positive immunohistochemical staining for vimentin, smooth-muscle-specific actin and desmin; stains for high- and low-molecular-mass cytokeratins, factor VIII-related antigen and leucocyte common antigen were negative (antibodies purchased from DAKO Corp., Carpinteria, CA, U.S.A.). Murine renal tubular epithelial cells were kindly provided by Dr Jeffery Kopp (National Institutes of Health, Bethesda, MD, U.S.A.). These cells were characterized by immunohistochemistry as keratin-positive, smooth-muscle-actin-negative, desmin-negative and vimentin-negative. Subcutaneous fibroblasts were grown from explants of dermal tissue obtained from Sprague–Dawley rats. A7r5 cells, H9C2 cells, LLCPK1 cells and HL-60 cells were obtained from the American Type Culture Collection (A.T.C.C., Rockville, MD, U.S.A.). Mesangial cells were grown to confluence in complete Waymouth's medium supplemented with 20 % (v/v) fetal calf serum. Tubular epithelial cells were grown in renal epithelial cell growth medium (Clontech, Palo Alto, CA, U.S.A.). Other cell lines were grown in Dulbecco's modified Eagle's medium/F12 medium supplemented with 10 % (v/v) fetal calf serum, according to instructions provided by A.T.C.C.

Preparation of microsomes

Microsomes were prepared from cultured cells, as previously described [18,22]. In brief, harvested cells were suspended in a buffer containing 300 mM sucrose, 10 mM Hepes, 0.1 mM EDTA and 0.5 mM PMSF (pH 7.4) at 4 °C, and homogenized using a Polytron (3×30 s at setting 10). The homogenate was centrifuged for 10 min at 1600 ***g***, and the pellet was discarded. The supernatant was further centrifuged at 11000 ***g*** for 20 min, and the supernatant thus obtained was ultracentrifuged at 100000 ***g*** for 1 h. The pellet was resuspended in a small volume of homogenizing buffer using a Dounce homogenizer. The microsomes were then either used fresh for measurement of $^{45}Ca^{2+}$ release, or divided into aliquots, quickly frozen and stored at −80 °C for measurement of [^{3}H]ryanodine binding.

[^{3}H]Ryanodine binding

This was carried out as described in [23,24]. Microsomes (100–200 μg of protein) were incubated for 2 h at 37 °C in a medium containing (final concentrations) 600 mM KCl, 100 μM EGTA, 150 μM Ca^{2+}, 0.2 μM PMSF, 25 μM Hepes (pH 7.2) and 30 nM [^{3}H]ryanodine (54.7 Ci/mM) bound to microsomes by a rapid filtration technique using Whatman GF/B filters, followed by three subsequent washes in 3 ml of ice-cold water. Free [^{3}H]ryanodine was separated from [^{3}H]ryanodine bound to membranes. The [^{3}H]ryanodine radioactivity that remained on the filters was measured by liquid scintillation counting [23,24]. The high-affinity specific [^{3}H]ryanodine binding was calculated as the difference between total binding and non-specific binding, determined in the presence of a 3000-fold higher concentration of unlabelled ryanodine (0.1 mM) or Ruthenium Red (10 μM).

$^{45}Ca^{2+}$ release from microsomes

Freshly prepared microsomes (approx. 100 μg of protein) were loaded passively by incubation for 3 h at room temperature (21 °C) in a medium containing 100 mM NaCl, 25 mM Hepes (pH 7.2), 1 mM $CaCl_2$ and 1 μCi of $^{45}Ca^{2+}$. Release of $^{45}Ca^{2+}$ from loaded microsomes was initiated by 10-fold dilution of the microsomal suspension with a buffer containing 100 mM NaCl, 1 mM EGTA, 1 mM $MgCl_2$ and 25 mM Hepes (pH 7.2) [22]. After 10 s, the suspension was further diluted in medium of identical composition which also contained agonists or antagonists to be tested. $^{45}Ca^{2+}$ efflux was stopped 90 s after the second dilution with added test agents, and the $^{45}Ca^{2+}$ retained in the microsomes was separated from free $^{45}Ca^{2+}$ by a rapid filtration technique using Whatman GF/B filters. In previous studies, we found that NAADP elicits $^{45}Ca^{2+}$ release in a time-dependent manner [18]. Microsomal $^{45}Ca^{2+}$ release is linear with respect to time for periods greater than 90 s [18]. We therefore assessed microsomal $^{45}Ca^{2+}$ release after 90 s of incubation in the present study. The filters were rinsed three times in a solution containing 100 mM NaCl, 1 mM EGTA, 4 mM $MgCl_2$, 10 μM Ruthenium Red and 25 mM Hepes (pH 7.2). The $^{45}Ca^{2+}$ retained in microsomes was determined by liquid scintillation counting.

The protein content of fractions was determined by the method of Lowry et al. [25]. The results were evaluated statistically with the use of Student's *t*-test for group or paired comparison; P values of < 0.05 were considered statistically significant.

Synthesis of NAADP

The NAADP synthetic capacity was assessed as previously described [18]. In brief, membrane fractions of homogenized cells (0.3 mg/ml) were incubated with 0.2 mM β-NADP and 7 mM nicotinic acid at 37 °C for 30 min in a medium containing 0.25 M sucrose and 20 mM Tris/HCl (pH 6.5). The content of NAADP was determined using a sea urchin egg homogenate calcium release bioassay [13,26].

Sea urchin egg homogenate bioassay

Homogenates from sea urchin (*Lytechinus pictus*) eggs were prepared as described previously [13]. Frozen homogenates were thawed in a 17 °C water bath and diluted to 1.25 % (v/v) in a medium containing 2 units/ml creatine kinase, 4 mM phosphocreatine, 1 mM ATP and 3 μM Fluo-3. Fluo-3 fluorescence was monitored at 490 nm excitation and 535 nm emission in a 250 μl cuvette at 17 °C in a circulating water bath with continuous mixing by a magnetic stirring bar, using a Hitachi spectrofluorimeter (F-2000) [13,26].

RESULTS AND DISCUSSION

NAADP induces calcium release from rat mesangial cell microsomes

NAADP induced calcium release in rat mesangial cell microsomes in a dose-dependent manner, with a half-maximal concentration

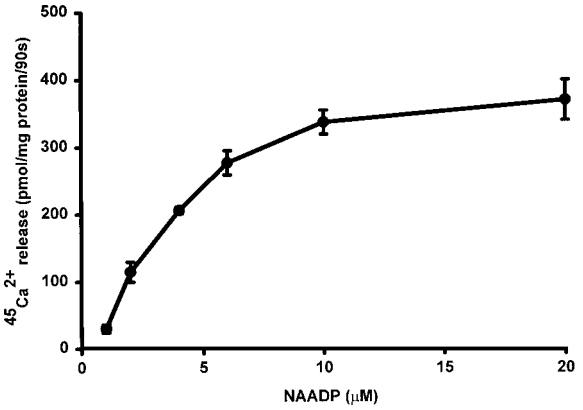

Figure 1 Dose-dependent effect of NAADP (1–20 μM) on calcium release from rat mesangial cell microsomes

Values are means ± S.E.M. Data are representative of three independent experiments.

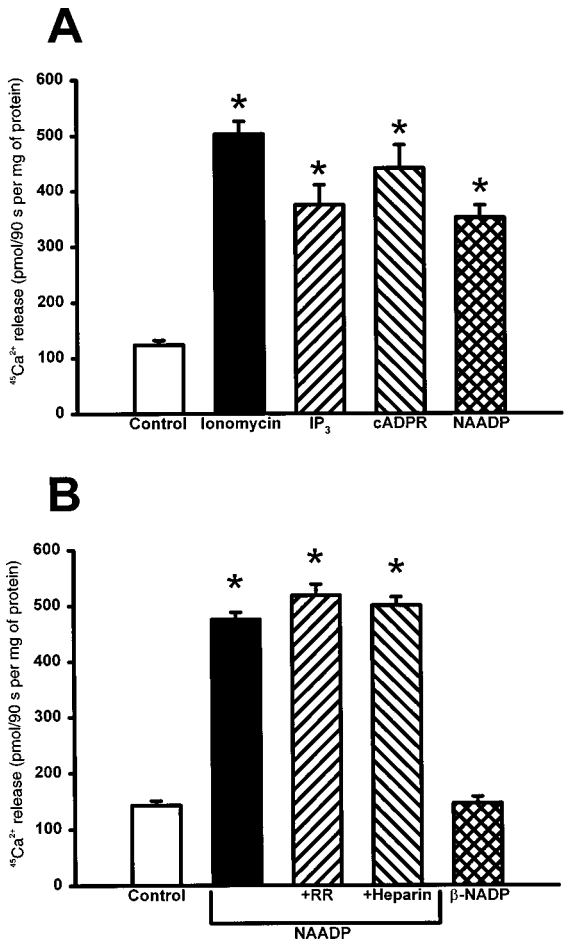

Figure 2 NAADP induces calcium release from rat mesangial cell microsomes

(**A**) Effects of various calcium-mobilizing agents on calcium release from mesangial cell microsomes. Microsomal release from control mesangial cells and from mesangial cells treated with 10 μM ionomycin, 8 μM IP_3, 10 μM cADPR or 10 μM NAADP was assessed as described in the Materials and methods section. Values are means $\pm$ S.E.M.; * indicates a significant difference between treated and control microsomes (*t*-test; $P < 0.05$). (**B**) Specific calcium release from rat mesangial cell microsomes. NAADP-induced calcium release from rat mesangial cell microsomes was determined as described in the Materials and methods section. Calcium release in untreated control microsomes was 160 $\pm$ 15 pmol/mg of protein. Treatments were: controls (no additions), 10 μM NAADP alone, 10 μM NAADP plus 10 μM Ruthenium Red (RR), 10 μM NAADP plus 1 mg/ml heparin, and 10 μM β-NADP. Values are means $\pm$ S.E.M.; * indicates a significant difference (*t*-test; $P < 0.05$) between treated and control microsomes.

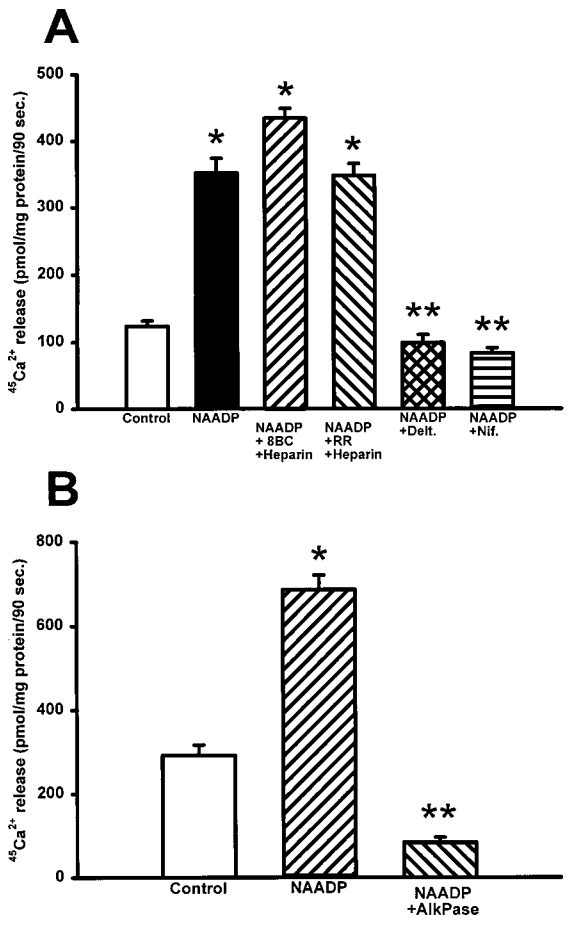

Figure 3 Specificity of NAADP-elicited calcium release from mesangial cell microsomes

(**A**) Effects of combined treatment with cADPR and IP_3 antagonists on NAADP-elicited $^{45}Ca^{2+}$ release from mesangial cell microsomes. $^{45}Ca^{2+}$ release from cells was assessed as described in the Materials and methods section. Treatments were: control, NAADP alone (10 μM), NAADP + 8-bromo-cADPR (8BC; 10 μM) + heparin (1 mg/ml), NAADP + Ruthenium Red (RR; 10 μM) + heparin (1 mg/ml), NAADP + diltiazem (Delt; 100 μM), and NAADP + nifedipine (Nif.; 100 μM). Values are means $\pm$ S.E.M.; * indicates a significant difference between treated and control microsomes ($P < 0.05$); ** indicates a significant difference between microsomes treated with NAADP alone and with NAADP plus a calcium channel blocker (diltiazem or nifedipine) (*t*-test; $P < 0.05$). (**B**) Alkaline phosphatase treatment of NAADP abolishes its ability to release calcium from mesangial cell microsomes. $^{45}Ca^{2+}$ release from mesangial cell microsomes was assessed as described in the Materials and methods section. Treatments were: control mesangial cell microsomes, microsomes treated with 10 μM NAADP, and microsomes treated with an identical NAADP solution (10 μM) that was incubated with alkaline phosphatase (AlkPase) (25 units/ml for 10 min at 35 °C) prior to treatment of microsomes. Values are means $\pm$ S.E.M.; * indicates a significant difference between treated and control microsomes; ** indicates a significant difference between microsomes treated with NAADP and with alkaline phosphatase-incubated NAADP (*t*-test; $P < 0.05$).

of 3 ± 1 μM (Figure 1). Addition of 10 μM NAADP to rat mesangial cell microsomes passively preloaded with $^{45}Ca^{2+}$ enhanced the rate of calcium efflux by 3-fold (Figure 2A). This induction was equivalent to approx. 70 % of the calcium release induced by ionomycin. The extent of calcium release elicited by NAADP was similar to that induced by cADPR or IP_3 (Figure 2A). Neither Ruthenium Red (a ryanodine channel inhibitor) nor heparin (an IP_3 inhibitor) had any significant effect on NAADP-mediated calcium release (Figure 2B) [9,27]. In contrast, β-NADP, the biosynthetic precursor of NAADP, did not induce significant calcium release (Figure 2B).

Blockade of both the cADPR and IP_3 calcium release systems by co-administration of 8-bromo-cADPR and heparin or Ruthenium Red and heparin had no effect on NAADP-elicited $^{45}Ca^{2+}$ release (Figure 3A). These studies exclude calcium-induced calcium release mediated by IP_3 or cADPR as potential contributors to NAADP-induced $^{45}Ca^{2+}$ release. In sea urchin egg homogenates [28,29] and in brain microsomes [16], L-type calcium channel blockers selectively block NAADP-elicited calcium release, but do not affect calcium-induced calcium release elicited by either IP_3 or cADPR. We found that two structurally distinct L-type calcium channel blockers, diltiazem and nifedipine, both abolished NAADP-elicited calcium release from mesangial cell microsomes (Figure 3A). Pretreatment of NAADP with alkaline phosphatase (25 units/ml) at 35 °C for 10 min

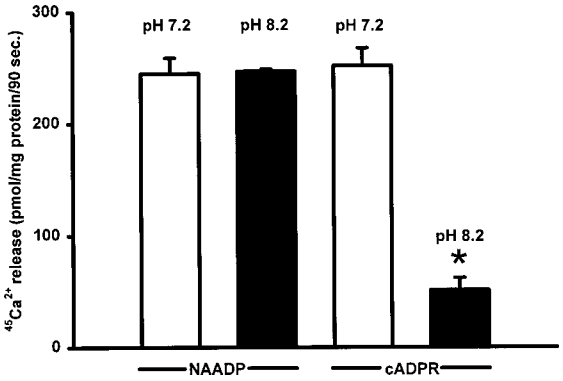

Figure 4 Effect of pH on calcium release elicited by NAADP and cADPR from rat mesangial cell microsomes

Calcium release from microsomes treated with 10 μM NAADP or 10 μM cADPR at pH 7.2 or 8.2 was assessed as described in the Materials and methods section. Data represent NAADP-specific calcium release (calcium release in the presence of NAADP minus calcium release in controls, which was 125 ± 4 pmol/90 s per mg of protein. Values are means $\pm$ S.E.M.; * indicates a significant difference (*t*-test; $P < 0.05$) from values observed at pH 7.2 for three separate experiments.

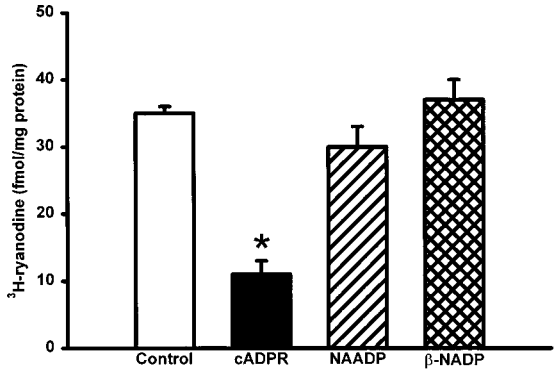

Figure 5 Effects of NAADP and cADPR on calcium-dependent [^{3}H]ryanodine binding to rat mesangial cell microsomes

[^{3}H]Ryanodine binding was measured in the presence of 50 μM calcium, as described in the Materials and methods section. cADPR, NAADP and β-NADP were each present at a concentration of 10 μM. Values are means $\pm$ S.E.M.; * indicates a significant difference from controls (*t*-test, $P < 0.05$). Data are representative of three independent experiments.

completely abolished its calcium-releasing ability (Figure 3B). These studies provide evidence that $^{45}Ca^{2+}$ release from mesangial cell microsomes is specific for NAADP and is not elicited by its biosynthetic precursor (β-NADP) or its degradation product (nicotinic acid–adenine dinucleotide).

Effects of IP_3 and cADPR on NAADP-elicited calcium release from mesangial cell microsomes

To determine whether there are any interactions between NAADP-elicited $^{45}Ca^{2+}$ release and $^{45}Ca^{2+}$ release evoked by the cADPR or IP_3 pathways, mesangial cell microsomes were treated with NAADP alone or with NAADP in the presence of IP_3 or cADPR. A maximal stimulatory dose of NAADP alone (10 μM) significantly increased microsomal $^{45}Ca^{2+}$ release (352 ± 22 pmol/90 s per mg of protein) compared with untreated control microsomes (124 ± 8 pmol/90 s per mg; $P < 0.05$). Co-administration of maximal stimulatory doses of IP_3 (8 μM) and NAADP (10 μM) produced a modest (17%) increase in $^{45}Ca^{2+}$ release (412 ± 16 pmol/90 s per mg) compared with that induced by NAADP alone. However, there was no significant difference in microsomal $^{45}Ca^{2+}$ release elicited by a combination of NAADP (10 μM) and IP_3 (8 μM) when compared with that due to IP_3 (8 μM) alone (375 ± 36 pmol/90 s per mg). Similarly, co-administration of a maximal stimulatory dose of NAADP (10 μM) with a maximal stimulatory dose of cADPR (10 μM) increased $^{45}Ca^{2+}$ release by 31% (462 ± 31 pmol/90 s per mg) compared with the release obtained with NAADP alone; this release was similar to that elicited by cADPR alone (441 ± 42 pmol/90 s per mg). These studies indicate that there may be considerable overlap of the calcium pools released by NAADP, IP_3 and cADPR. Similar observations have been made in microsomes isolated from rat brain tissue [16].

Effect of pH on NAADP-induced calcium release

We, and others, have shown previously that NAADP-induced calcium release in sea urchin egg homogenates is not affected by changes in the pH of the medium [27,30]. In agreement with our findings in sea urchin egg homogenates, NAADP-induced calcium release in rat mesangial cell microsomes was not affected by changing the pH of the medium from 7.2 to 8.2 (Figure 4). In contrast, cADPR-induced calcium release was inhibited by alkalinization of the media (Figure 4).

Effect of NAADP on [^{3}H]ryanodine binding

We have shown previously that rat mesangial cells possess a functional ryanodine receptor, which is involved in cADPR-mediated intracellular calcium release [31]. To provide additional evidence that calcium signalling through NAADP and cADPR are distinct, we tested the effects of these compounds upon [^{3}H]ryanodine binding to rat mesangial cell microsomes. As expected, cADPR profoundly inhibited [^{3}H]ryanodine binding to rat mesangial cell microsomes (Figure 5). However, NAADP (or its biosynthetic precursor β-NADP) had no significant effect on [^{3}H]ryanodine binding to mesangial cell microsomes (Figure 5).

NAADP does not promote significant calcium release in renal tubular epithelial cell microsomes

Employing the sea urchin egg bioassay, we reported recently that rat glomeruli and mesangial cells have a much higher capacity for synthesis of both cADPR and NAADP than tubular epithelial cells [18,32]. To determine whether this difference in capacity for NAADP biosynthesis between mesangial cells and tubular epithelial cells is paralleled by a similar difference in microsomal calcium release activity, we assessed microsomal calcium release in primary cultures of renal tubular epithelial cells. Ionomycin, a non-specific calcium ionophore, induced calcium release in microsomes from renal tubular epithelial cells by over 5-fold (Figure 6). IP_3 induced a 3.5-fold increase in calcium release. cADPR produced a modest increase in calcium release, whereas an equal concentration of NAADP failed to elicit any significant increase in calcium release (Figure 6).

NAADP elicits calcium release in microsomes prepared from a variety of cells

NAADP-induced calcium release has been described in only a few mammalian cell systems, including brain microsomes and pancreatic acinar cells [16,33]. Here we demonstrate that NAADP, but not β-NADP, is able to activate a specific micro-

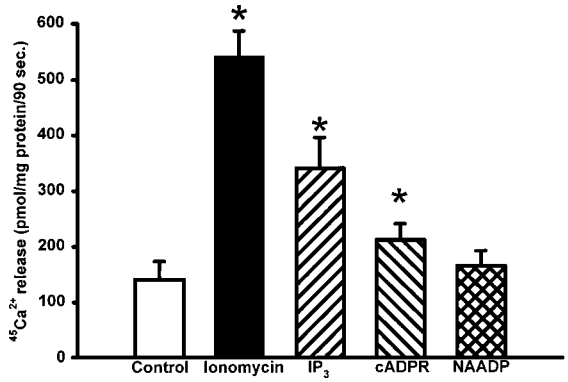

Figure 6 Effects of various calcium-mobilizing agents on calcium release from renal epithelial cell microsomes

Microsomal calcium release from control renal epithelial cells and from cells treated with 10 μM ionomycin, 10 μM IP_3, 10 μM cADPR or 10 μM NAADP was assessed as described in the Materials and methods section. Values are means $\pm$ S.E.M.; * indicates a significant difference between treated and control microsomes (*t*-test; $P < 0.05$).

somal calcium release system in several mammalian cell types, including vascular smooth muscle cells (A7r5), cardiac myocytes (H9C2), subcutaneous fibroblasts and a human leukaemia cell line (HL-60) (Figure 7A). In these cells, calcium release (pmol/90 s per mg of protein) is one-third to one-half that elicited by the non-specific ionophore ionomycin.

NAADP did not promote significant calcium efflux from microsomes isolated from LLCPK1 cells, a renal tubular epithelial cell line. This observation is in accordance with results from primary renal tubular epithelial cell cultures (Figure 6). Based on these findings, we propose that NAADP-induced calcium release in microsomes is cell-type-specific, and is probably not due to a non-specific ionophoric effect.

NAADP-elicited microsomal calcium release does not always correlate with capacity for biosynthesis of NAADP in membrane extracts

NAADP synthetic capacity was determined for the cell lines shown in Figure 7(A). Membrane fractions from the cell lines were incubated with 0.2 mM β-NADP and 7 mM nicotinic acid for 30 min. NAADP production was determined by the sea urchin egg homogenate bioassay, as described in the Materials and methods section. Rat mesangial cells, which have an active NAADP-elicited microsomal calcium release system (Figures 1–4 and 6), have a high capacity for NAADP synthesis, as described previously [18] (Figure 7B). Primary cultures of renal tubular epithelial cells (WT) have no significant capacity for NAADP biosynthesis (Figure 7B) and show minimal NAADP-elicited microsomal calcium release activity (Figure 6). Subcutaneous fibroblasts, the A7r5 smooth muscle line and the HL-60 monocyte cell line are endowed with both NAADP biosynthetic capacity (Figure 7B) and the capacity for NAADP-elicited microsomal calcium release (Figure 7A). However, the capacity for NAADP synthesis did not, in all cases, correlate with NAADP-induced microsomal calcium release. For example, LLCPK1 cells, an epithelial-like cell line derived from pig kidney [34], have a high capacity for NAADP biosynthesis, but microsomes from LLCPK1 cells lack the ability to release calcium in response to NAADP. Microsomes isolated from the H9C2 cardiac myocyte cell line release calcium in response to NAADP

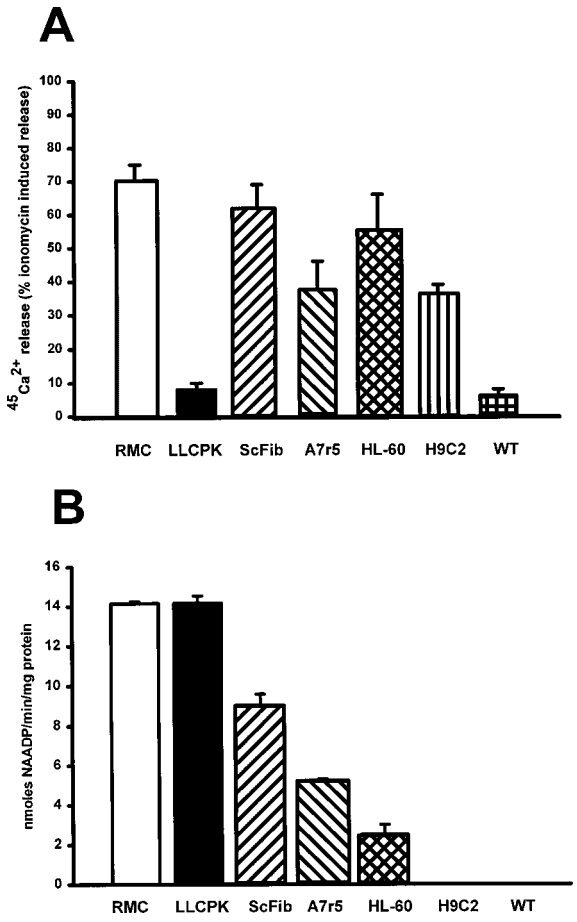

Figure 7 Relationship between NAADP-elicited calcium release and capacity for NAADP synthesis in mammalian cells

(**A**) NAADP promotes $^{45}Ca^{2+}$ release in a variety of mammalian cell types. Results are means $\pm$ S.E.M. ($n = 3$ independent experiments), and are expressed as a percentage of ionomycin-induced calcium release. NAADP-specific calcium release ($^{45}Ca^{2+}$) in the presence of NAADP minus $^{45}Ca^{2+}$ release in untreated controls was (pmol/90 s per mg of protein): 352 ± 22 for rat mesangial cells (RMC), 43 ± 15 for LLCPK cells, 379 ± 55 for subcutaneous fibroblasts (ScFib), 257 ± 40 for A7r5 cells, 298 ± 108 for HL-60 cells, 302 ± 56 for H9C2 cells and 24 ± 9 for renal tubular epithelial cells (WT). (**B**) Capacity for NAADP synthesis in mammalian cells. NAADP synthetic capacity was assessed in membrane fractions of the indicated cell types, as described in the Materials and methods section. NAADP content was measured by the sea urchin egg homogenate bioassay (see the Materials and methods section). Data are representative of three independent experiments, each performed in triplicate.

(Figure 7A), but lack the capacity for NAADP biosynthesis (Figure 7B).

These studies demonstrate for the first time, to our knowledge, that there may be a discordance between NAADP-elicited microsomal calcium release and the capacity for NAADP biosynthesis in mammalian cells. Although the putative NAADP receptor/channel has not yet been isolated or characterized in mammalian cells, the microsomal calcium release assay results (Figure 7A) provide evidence that it may be present in many mammalian cells and tissues. The capacity for NAADP biosynthesis is indicative of ADP-ribosyl cyclase activity [15,28,35]. However, relatively high concentrations of β-NADP and nicotinic acid are required to demonstrate NAADP biosynthesis in mammalian cells. The physiological relevance of the synthetic pathway to calcium homoeostasis in normal mammalian cells and tissues remains to be established.

Conclusions

In summary, we provide evidence that microsomes isolated from several histologically distinct mammalian cell types contain a calcium release system that is specifically induced by NAADP. NAADP-induced calcium release has several unique properties, including: (1) it is not affected by bivalent cations such as calcium or magnesium [27,28,36]; (2) it is not affected by changes in the physiological pH range [27,28,36]; and (3) it can be self-inactivated by low concentrations of NAADP [28,29,37]. These properties make NAADP a novel and unique intracellular calcium-releasing agent. Our present observations suggest that the NAADP calcium signalling system may be widespread in mammalian cells and tissues, and may prove to be an important mediator of intracellular calcium signalling. Future studies are needed to characterize the NAADP receptor/channel in mammalian cells, and to define physiologically relevant biosynthetic pathways for the production of NAADP, or perhaps for other ligands that may activate this receptor/channel.

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