Differential effect of pH upon cyclic-ADP-ribose and nicotinate–adenine dinucleotide phosphate-induced Ca^{2+} release systems

Eduardo N. CHINI¹, Mingyu LIANG and Thomas P. DOUSA

Mayo Clinic and Foundation, Department of Physiology and Biophysics and Department of Anesthesiology, 200 First Street, SW, 901 Guggenheim Bldg., Rochester, MN 55905, U.S.A.

We investigated the pH dependence and the effects of thimerosal and dithiothreitol (DTT) upon the Ca^{2+} release induced by cADP-ribose (cADPR) and nicotinate–adenine dinucleotide phosphate (NAADP) in sea urchin egg homogenates. Both Ca^{2+} release triggered by cADPR and the binding of [³H]cADPR to sea urchin egg homogenates were decreased by alkalization of the assay media from pH 7.2 to 8.9. In contrast, NAADP-triggered Ca^{2+} release was not influenced by changes in pH. The

Ca^{2+} release induced by cADPR was potentiated by thimerosal and inhibited by DTT, but neither thimerosal nor DTT had any effect upon the Ca^{2+} release induced by NAADP. We conclude that cADPR-sensitive Ca^{2+} -release mechanisms are dependent on pH of the assay media and are sensitive to thiol group modification. On the other hand, these functional properties are not shared by NAADP-regulated Ca^{2+} channels.

INTRODUCTION

Release of Ca^{2+} from intracellular stores is an important component in several signalling pathways [1,2]. In sea urchin eggs, a transient rise in intracellular Ca^{2+} concentration occurs at the sperm–egg fusion site during fertilization. Two major mechanisms of intracellular Ca^{2+} release in sea urchin eggs are now well characterized: (i) the inositol 1',4',5'-trisphosphate (IP_3)-triggered Ca^{2+} release [1,2]; and (ii) the so-called Ca^{2+} -induced Ca^{2+} -release (CICR) system, mediated by the ryanodine receptor/channel (RyR). RyR is activated by the endogenous nucleotide cADP-ribose (cADPR) [3–5]; however, the mechanism of activation of the RyR by cADPR in sea urchin egg homogenates is not completely understood. In particular, whether cADPR binds directly to the RyR or acts indirectly is unclear. According to a current view, cADPR may bind to a receptor protein non-covalently associated with RyR and thereby activates Ca^{2+} release through the RyR [4,5].

In addition to the Ca^{2+} release induced by cADPR and IP_3 , sea urchin eggs possess another Ca^{2+} releasing system that is activated by nicotinate–adenine dinucleotide phosphate (NAADP), a recently discovered potent stimulator of intracellular Ca^{2+} release [6–9]. NAADP-induced Ca^{2+} release was demonstrated in both homogenates and intact sea urchin eggs [6–15]. The Ca^{2+} release mechanism triggered by NAADP is different in many ways from those controlled by IP_3 and cADPR [6–15]. Unique properties of the NAADP-induced Ca^{2+} -release mechanism include: (i) the absence of cross-desensitization with IP_3 and cADPR; and (ii) the lack of inhibition of NAADP-induced Ca^{2+} release by known antagonists of cADPR and IP_3 . We have described previously how NAADP-induced Ca^{2+} release is not involved in the CICR mechanism [10]. In addition, NAADP-induced Ca^{2+} release is not affected by divalent cations, such as Ca^{2+} , Mg^{2+} and Sr^{2+} [10–15]. Furthermore, NAADP-induced Ca^{2+} release in sea urchin eggs is specifically inhibited by L-type

Ca^{2+} channel blockers [13]. However, the mechanism of Ca^{2+} release induced by NAADP is not yet fully determined.

During fertilization of sea urchin eggs, an increase of intracellular pH is observed; indeed, the cytoplasmic pH becomes 0.4 units more alkaline after fertilization [16], and this change *in vivo* in pH may modulate Ca^{2+} release from intracellular stores. It has been reported previously that Ca^{2+} release through intracellular Ca^{2+} channels can be modulated by pH and thiol-reactive compounds [17–23]. In view of this, we explored the effect of pH, and some thiol-reactive compounds, upon Ca^{2+} release induced by cADPR, IP_3 and NAADP in sea urchin egg homogenates. We found that the NAADP-induced Ca^{2+} release mechanism is modulated neither by the change of pH nor by thiol-reactive compounds. On the other hand, cADPR-induced Ca^{2+} release, but not IP_3 -induced Ca^{2+} release, is decreased in response to alkaline pH; cADPR-triggered Ca^{2+} release is potentiated by the thiol-reactive compound thimerosal. These unique functional properties suggest that NAADP-triggered Ca^{2+} release is probably mediated via a new type of channel, which is different from the IP_3 - and cADPR-regulated RyR channels.

METHODS AND MATERIALS

Sea urchin egg homogenates

Homogenates from *Lytechinus pictus* eggs were prepared as described previously [6], with minor modifications. Eggs were obtained by injection of 0.5 M KCl into the coelomic cavity, and shed into artificial sea water. The jelly coats were washed from the eggs by several passages through 80- μm -mesh silk. The eggs were then washed once in artificial sea water, twice in Ca^{2+} -free sea water containing 1 mM EGTA, twice in Ca^{2+} -free water without EGTA and once in a homogenizing buffer containing 250 mM *N*-methyl glucamine, 250 mM potassium gluconate,

Abbreviations used: cADPR, cADP-ribose; NAADP, nicotinate–adenine dinucleotide phosphate; IP_3 , inositol 1',4',5'-trisphosphate; RyR, ryanodine receptor/channel; CICR, Ca^{2+} -induced Ca^{2+} -release; IM, intracellular medium.

¹ To whom correspondence should be addressed.

20 mM Hepes buffer (pH 7.2), 1 mM MgCl_2 , 2 units/ml creatine kinase, 4 mM phosphocreatine, 1 mM ATP, 25 $\mu\text{g/ml}$ leupeptin, 20 $\mu\text{g/ml}$ aprotinin and 100 $\mu\text{g/ml}$ soya-bean trypsin inhibitor. A 25% suspension was prepared by homogenization with 4–5 strokes in a Dounce homogenizer with a type-A pestle, the homogenate was then centrifuged for 10–12 s at 13000 g at 4 °C. The supernatant was collected and stored in 1 ml aliquots at –70 °C. Frozen homogenates were thawed in a 17 °C water bath and diluted to 1.25% with an intracellular medium (IM) solution containing 250 mM *N*-methyl glucamine, 250 mM potassium gluconate, 20 mM Hepes buffer (pH 7.2), 1 mM MgCl_2 , 2 units/ml creatine kinase, 4 mM phosphocreatine, 1 mM ATP, 3 $\mu\text{g/ml}$ oligomycin and 3 $\mu\text{g/ml}$ antimycin. After incubation at 17 °C 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 at 17 °C with a circulation water bath and continuously mixed with a magnetic stirring bar, using a Hitachi spectrofluorimeter (F-2000). The stock solutions of various substances that were added did not exceed 2% of the homogenate volume in the cuvette.

Ca^{2+} -release assay

For the Ca^{2+} -release experiments, sea urchin egg homogenates were loaded with Ca^{2+} for 3 h as described above. The release of Ca^{2+} triggered by the different agents was determined after pretreatment of the homogenate for 2 min with 0–24 mM KOH, NaOH or HCl to obtain the desired final pH. The final pH was determined in control experiments. In none of the experimental conditions did the change in pH by itself induce significant release of Ca^{2+} . In addition, neither NaCl nor KCl up to 25 mM had any effect upon Ca^{2+} release induced by the agents tested (results not shown).

Binding of cADPR

Binding of cADPR was performed as described previously [6] using [^3H]cADPR. Sea urchin egg homogenates (2 mg/ml) were diluted in IM with the addition of 1 mM EGTA and incubated with different concentrations of [^3H]cADPR (100 d.p.m./fmol) for 10 min on ice. After incubation, the mixture (100 μg of protein) was filtered through prewashed fibreglass (Whatman Co.) under vacuum and rapidly washed twice with 2 ml of ice-cold IM. Radioactivity retained on the filters was determined using standard scintillation-counting techniques.

Preparation of nucleotides

cADP-ribose was synthesized by *Aplysia* ADP-ribosyl cyclase [24] using homogenized *Aplysia* ovotestes as described previously [6]. NAADP was synthesized via the base-exchange reaction catalysed by NAD-glycohydrolase with minor modifications [6]. Nicotinic acid (100 mM) and β -NADP $^+$ (10 mM) were incubated in 20 mM triethanolamine (pH 7.6) with 0.2 g/ml porcine brain NAD(P)ase (Sigma Co.; St. Louis, MO, U.S.A.) at 37 °C for 3 h. The reaction was stopped by acetone precipitation as described above for synthesis of cADPR. Neither the spontaneous hydrolysis of cADPR nor the hydrolysis catalysed by the sea urchin egg homogenate were increased when the pH of the media was changed from 6.7 to 8.9 (results not shown) [25].

After acetone preparation, the nucleotides were purified by HPLC anion-exchange chromatography using AG MP-1 resin (Bio-Rad; Hercules, CA, U.S.A.) packed into a 1 $\times$ 10 cm column. The nucleotides were eluted with a non-linear gradient of 150 mM trifluoroacetic acid and water, and monitored by UV absorption

at 250 nm. Purified cADPR or NAADP were evaporated to dryness in a SpeedVac concentrator. cADP-ribose and NAADP used in all experiments were at least 97% pure as determined by HPLC.

Materials

L. pictus and *Aplysia californica* were obtained from Marinus, Inc., (Long Beach, CA, U.S.A.). Fluo-3 was purchased from Molecular Probes (Eugene, OR, U.S.A.); IP $_3$, ryanodine, oligomycin and antimycin were from Calbiochem (La Jolla, CA, U.S.A.). [^3H]cADPR was from Amersham (Arlington, IL, U.S.A.). All other reagents, of the highest-purity grade available, were supplied by Sigma.

The reported experiments were repeated at least 3–6 times. When appropriate, the results were analysed statistically using a group *t*-test.

RESULTS

Effect of pH upon Ca^{2+} -release

In the present study we determined how intracellular Ca^{2+} release induced by three different agents, NAADP, IP $_3$ and cADPR, was affected by changes in pH of the assay media ranging from 6.7 to 8.9 (Figure 1). Progressive increase of pH inhibited the cADPR-induced Ca^{2+} -release system; on the other hand, Ca^{2+} release induced by IP $_3$ or NAADP was not influenced by changes in pH. As shown in Figures 1 and 2, cADPR-induced Ca^{2+} release was nearly abolished when the pH was increased from 6.7 to 8.9. Moreover, alkalization of the media could also block the Ca^{2+} release induced by cADPR after the release was initiated. Shortly after the addition of 25 nM cADPR, which triggered Ca^{2+} release, addition of NaOH to the assay media, changing the pH from 7.2 to 8.0, was able to reduce the rate of ongoing Ca^{2+} release (Figure 2D). Furthermore, the effect of alkalization was reversible. As shown in Figures 2(B) and 2(C), when the pH that had been adjusted to 8.0 beforehand was decreased to 7.2 by the addition of HCl, the Ca^{2+} -release activity of cADPR was restored. The comparison of concentration-dependence curves for cADPR-induced Ca^{2+} release at pH 7.2 and 8.0 shows a decrease

Figure 1 Effect of pH upon Ca^{2+} release in sea urchin egg homogenates

The figure shows the net rate of Ca^{2+} release induced by 1 μM IP $_3$ (□), 100 nM cADPR (●) and 100 nM NAADP (△) at different pHs. The data represent the means $\pm$ S.E.M. of 4 experiments.

Figure 2 Reversible effect of pH upon cADPR-induced Ca^{2+} release

Ca^{2+} release in 1.25% sea urchin egg homogenates was monitored by fluo-3 as a Ca^{2+} indicator. The initial pH before addition of the agents was 7.2. Homogenates were treated with the following additions as shown in the Figure: 25 nM cADPR; 12 mM NaOH (to obtain final pH 8.0); 12 mM NaOH and 12 mM HCl (to reduce the pH back to 7.2). In (B), + indicates addition of HCl (final pH 7.2); in (D), + indicates the addition of NaOH (final pH 8.0).

in the apparent affinity for cADPR by alkalization (Figure 3A). The decrease of cADPR-triggered Ca^{2+} release by alkalization of the media may be due to a decrease of the binding affinity of cADPR to its receptor. It was described previously that the binding of cADPR is inhibited by alkalization of the media [26]. As shown in Figure 4(A), the binding of cADPR to the sea urchin egg homogenate was inhibited by an increase of the assay media pH. Furthermore, the apparent affinity for cADPR binding was shifted from ≈ 15 nM at pH 7.2 to higher than 20 nM at pH 8 (Figure 4B); at pH 8, maximum binding of cADPR was suppressed by $\sim 70\%$. It was proposed previously that cADPR acts on the RyR indirectly by first binding to a specific receptor [4,5,12,27], whereas other agonists can activate the RyR directly [12]. In view of this we also explored the effect of alkalization of the pH on the Ca^{2+} release induced by the agonist of the RyR channel, caffeine [11,28,29]. In these experiments the Ca^{2+} -release agonist was used in concentrations twice the half-maximum activating concentration, determined in preliminary experiments. We found that, akin to cADPR, the Ca^{2+} release induced by caffeine was also inhibited by alkalization of the pH (Figure 5), but to a significantly less degree than that of the Ca^{2+} release induced by cADPR (Figure 5).

cADPR-induced Ca^{2+} release can be potentiated by caffeine [28,29]. As described above, in alkaline pH 8.9, Ca^{2+} release triggered by cADPR was suppressed. However, the addition of 2 mM caffeine, which did not cause release Ca^{2+} by itself (Figure 6A), to sea urchin egg homogenates at pH 8.9 counteracted the effect of alkalization upon the cADPR-induced Ca^{2+} release (Figure 6B). These data indicate that the RyR agonists can prevent the inhibitory effect of alkalization upon cADPR-induced Ca^{2+} release.

Figure 3 Dose-dependence of Ca^{2+} -releasing agents

(A) Dose-dependence for cADPR, (B) dose-dependence for NAADP. The experiment was carried out as described in the Methods and materials section. The Ca^{2+} release induced by different concentrations of the Ca^{2+} -release agents was tested in pH 7.2 (●) or pH 8.0 (□). The data are representative of the means $\pm$ S.E.M. of 3 independent experiments.

Effect of pH upon NAADP-induced Ca^{2+} release

In contrast with cADPR, NAADP-induced Ca^{2+} release was totally independent of the pH of the media range tested (pH 6.7–8.9) (Figure 1). This independence of pH was observed for Ca^{2+} release induced by NAADP in the concentration range 15–100 nM (Figure 3B).

Effect of thimerosal upon cADPR-induced Ca^{2+} release

It was demonstrated previously that thimerosal, a compound that blocks thiol groups, can enhance the Ca^{2+} release elicited by both IP_3 and cADPR in sea urchin egg homogenates [22,23]. As shown in Figure 7, thimerosal shifted the dose-response curve for cADPR-induced Ca^{2+} release to the left. In addition, we observed that thimerosal is also able to potentiate the Ca^{2+} release induced by other agonists of the RyR, such as caffeine (Figure 7B). We also observed that thimerosal has no effect upon $[^3\text{H}]$ cADPR binding, which suggests that thimerosal does not interact directly with the cADPR receptor (results not shown). We propose that thimerosal acts directly upon the RyR to increase RyR sensitivity to activators, but with no effect upon the cADPR receptor.

Effect of thimerosal and dithiothreitol upon NAADP-induced Ca^{2+} release

We also tested the effect of thimerosal upon Ca^{2+} release induced by NAADP. We found that thimerosal, up to 50 μM , had no

Figure 4 Inhibition of the cADPR binding

(A) The effect of pH upon binding of 40 nM [^3H]cADPR to the sea urchin egg homogenates. (B) Dose-dependence of cADPR binding at pH 7.0 (●) and pH 8.0 (○). Binding of cADPR was determined as described in the Methods and materials section. The data represent means $\pm$ S.E.M. of 3 independent experiments.

Figure 5 Effect of pH upon Ca^{2+} release in sea urchin egg homogenates

The net rate of Ca^{2+} release induced by 80 nM cADPR (●) or 4 mM caffeine (□) in different pHs. The data represent the means $\pm$ S.E.M. of 4 experiments. * Indicates significant differences between the percentage of Ca^{2+} release induced by cADPR and caffeine.

effect upon this Ca^{2+} release (Figure 7C). In addition, we observed that the reducing agent dithiothreitol inhibited about 50 % of the Ca^{2+} release induced by cADPR, but had no effect upon Ca^{2+} release induced by NAADP (results not shown).

Figure 6 Reversal of the alkalization effect on cADPR-induced Ca^{2+} release by caffeine

Ca^{2+} release in 1.25% sea urchin egg homogenates was monitored by fluo-3 as a Ca^{2+} indicator. The initial pH before the addition of the agents was 7.2; the homogenates were pretreated with 12 mM NaOH (to obtain final pH 8.0). After adjusting the pH to 8.0, the homogenates were treated with 40 nM cADPR and 2 mM caffeine as shown in the Figure.

DISCUSSION

Shen and Buck, using fluorescent pH probes, estimated an increase in the egg intracellular pH from 6.84 to 7.22 during the first minutes after fusion of the sperm and egg [30]. Cytoplasmic egg alkalization is the consequence of activation of the $\text{Na}^+\text{-H}^+$ antiporter, which may be regulated either by protein kinase C or by Ca^{2+} -calmodulin-dependent protein kinase [31]. The increase in intracellular pH after fertilization appears to be fundamental for metabolic activation of the sea urchin egg [19,32].

It was proposed that the ionic fluxes during fertilization of sea urchin eggs are mediated by several second-messenger pathways, including the ones involved in mobilization of intracellular Ca^{2+} [4,5,19]. However, whether the change *in vivo* in pH modulates the intracellular Ca^{2+} mobilization in sea urchin egg homogenates is still an open question. It was proposed previously that in the sea urchin, the sperm guanylyl cyclase could be activated by the acidic intracellular pH of the egg [32]. The local increase in cGMP, sustained enzymically until the intracellular pH of the egg increases, would stimulate the synthesis of cADPR by cytosolic cADPR-cyclase and thereby trigger the release of intracellular Ca^{2+} [32,33].

In addition, it is possible that the alkalization of the intracellular pH during fertilization is an important regulator of the intracellular Ca^{2+} channels. It has been reported previously that Ca^{2+} release through intracellular Ca^{2+} channels can be regulated by pH [17–19]. Here we report the effect of alkalization of the pH upon the Ca^{2+} release mediated by the three known modulators of intracellular Ca^{2+} release in sea urchin egg homogenates. We found that alkalization of the assay media had a profound inhibitory effect upon cADPR-induced Ca^{2+} release. Finally, the Ca^{2+} release induced by NAADP appears to be completely independent of the assay-media pH in a wide range of pHs and NAADP concentrations.

We propose that alkalization of the media have effect upon both binding of cADPR to its receptor and direct activation of the RyR channel by pharmacologic agonists. According to a recent report, alkalization of medium had, in contrast with our observations (Figures 1 and 2), only a minor inhibitory effect

Figure 7 Effect of thimerosal upon Ca^{2+} release induced by cADPR, caffeine and NAADP

Ca^{2+} release was determined as described in the Methods and materials section. The sea urchin egg homogenate was preincubated with 50 μM thimerosal for 1 min prior to the addition of the Ca^{2+} -releasing agents. In our sea urchin egg homogenate preparation, this concentration of thimerosal promoted no significant Ca^{2+} release by itself. Concentrations higher than this were able to induce Ca^{2+} release and were not tested for potentiation of the Ca^{2+} release. Concentrations up to 50 μM had no effect upon the Ca^{2+} release induced by NAADP. Dose-dependence of the Ca^{2+} -release agent was determined without (□) or with (●) preincubation with thimerosal. The data represent the means $\pm$ S.E.M. of 3 independent experiments.

upon cADPR-induced Ca^{2+} release [34]. This discrepancy between these data may be only due to the facts that Genazzani et al. tested the effect of pH alkalization upon Ca^{2+} release induced by saturating concentrations of cADPR [34] and, as demonstrated in Figure 3, the apparent affinity for cADPR is decreased by alkalization. We observed that in contrast with cADPR, the Ca^{2+} release induced by NAADP is not modulated by a change in pH in the range tested. We propose that protonation and

deprotonation of relevant amino acids with pK values in the range of physiological pH has no effect upon the gating property of the putative channel that is activated by NAADP and, by extension, the binding of NAADP to its receptor. Recent data by Genazzani et al. are in accord with this observation [34].

Furthermore, it was proposed previously that oxidation of critical thiol groups within the RyR and IP_3 channels is important to control their Ca^{2+} -gating properties [19–23,35–38]. In contrast to these channels, the NAADP-induced Ca^{2+} release is not modulated by either oxidation or reduction of thiol groups. These observations may provide the first indication of a structural difference between NAADP-induced Ca^{2+} -release mechanism and that through the RyR and IP_3 channels. The NAADP-induced Ca^{2+} release has several other unique features that differentiates it from the previously described mechanisms of intracellular Ca^{2+} release [6–15,39–41]. In our previous studies we have shown that NAADP-induced Ca^{2+} release lacks the Ca^{2+} feedback and, unlike cADPR and IP_3 -triggered Ca^{2+} release, does not behave as a 'functional' CICR system [10]. In addition, the NAADP-induced Ca^{2+} release is fully inactivated by exposure to low concentrations of NAADP [13,39–41]. These observations indicate that NAADP-induced Ca^{2+} release is mediated by a unique type of Ca^{2+} -release channel. Taken together, the sensitivity to changes in Ca^{2+} and pH can be another criterion that differentiates Ca^{2+} release in response to cADPR, IP_3 and NAADP.

In conclusion, our results indicate that a rise in intracellular pH may be an endogenous modulator of Ca^{2+} release during fertilization. The reported change in intracellular pH from 6.8 to 7.2 during fertilization could therefore modulate both cADPR- and IP_3 -induced Ca^{2+} release by, as demonstrated here, activating the IP_3 -induced Ca^{2+} -release system and inhibiting cADPR-induced Ca^{2+} release. We have proposed previously a similar role for polyamines upon cADPR-induced Ca^{2+} release during fertilization of sea urchin eggs [8].

In addition to the Ca^{2+} wave associated with egg fertilization, multiple Ca^{2+} transients subsequently occur in the sea urchin egg [42]. The timing of these latter Ca^{2+} transients correlates with various developmental events [42]. Mechanisms for these late Ca^{2+} waves are not well known. It is conceivable that an increase in intracellular pH and the level of polyamines (1–2 mM by 30 min) after fertilization may counteract the effect of cADPR as a Ca^{2+} mobilizing factor, especially in these late Ca^{2+} transients, and IP_3 would then be a major regulator of these phenomena [8,43]. Indeed, there is evidence that IP_3 levels fluctuate during the cell cycle [44] and may be involved in the regulation of intracellular Ca^{2+} during the late Ca^{2+} oscillations in sea urchin eggs [44]. However, whether this change *in vivo* in pH modulates the Ca^{2+} transient during fertilization, as proposed here, is an issue that needs further evaluation. Of particular interest is the role of the pH-insensitive Ca^{2+} release induced by NAADP during fertilization.

This research was supported in part by the American Heart Association (Minnesota Affiliate) grant-in-aid to E.N.C., NIH grants DK-30579 and DK-16105, and by the Mayo Foundation. E.N.C. was a research fellow of the National Kidney Foundation. The authors would like to thank Claudia C. S. Chini and Chad Bolliger for the critical reading of the manuscript.

REFERENCES

- Berridge, M. J. (1994) *Mol. Cell. Endocrinol.* **98**, 119–124
- Clapham, D. E. (1995) *Cell* **80**, 259–268
- Coronado, R., Morrisette, J., Sukhareva, M. and Vaughan, D. M. (1994) *Am. J. Physiol.* **266**, C1485–C1504
- Lee, H. C. (1994) *Mol. Cell. Biochem.* **138**, 229–235
- Gallone, A. and White, A. (1994) *Trends Cell. Biol.* **4**, 431–436

- 6 Chini, E. N., Beers, K. W. and Dousa, T. P. (1995) *J. Biol. Chem.* **270**, 3216–3223
- 7 Lee, H. C. and Aarhus, R. A. (1995) *J. Biol. Chem.* **270**, 2152–2157
- 8 Chini, E. N., Beers, K. W., Chini, C. S. and Dousa, T. P. (1995) *Am. J. Physiol.* **269**, C1042–C1047
- 9 Perez-Terzic, C. M., Chini, E. N., Shen, S. S., Dousa, T. P. and Clapham, D. E. (1995) *Biochem. J.* **312**, 955–959
- 10 Chini, E. N. and Dousa, T. P. (1996) *Biochem. J.* **316**, 709–711
- 11 Chini, E. N. and Dousa, T. P. (1996) *Am. J. Physiol.* **270**, C530–C537
- 12 Dousa, T. P., Chini, E. N. and Beers, K. W. (1996) *Am. J. Physiol.* **271**, C1007–C1024
- 13 Genazzani, A. A. and Galione, A. (1997) *Trends Pharmacol. Sci.* **18**, 108–110
- 14 Genazzani, A. A. and Galione, A. (1996) *Biochem. J.* **315**, 721–725
- 15 Graeff, R. M., Podein, R. J., Aarhus, R. and Lee, H. C. (1995) *Biochem. Biophys. Res. Commun.* **206**, 786–791
- 16 Epel, D. (1990) *Cell. Diff. Dev.* **29**, 1–12
- 17 Worley, P. F., Baraban, J. M., Supattapone, S., Wilson, V. and Snyder, S. H. (1987) *J. Biol. Chem.* **262**, 12132–12136
- 18 Iino, M. (1989) *J. Gen. Physiol.* **94**, 363–383
- 19 Guillemette, G. and Segui, J. A. (1988) *Mol. Endocrinol.* **2**, 1249–1255
- 20 Hilly, M., Pietri-Rouxel, F., Coquil, J. F., Guy, M. and Mauger, J. P. (1993) *J. Biol. Chem.* **268**, 16488–16494
- 21 Sayers, L. G., Brown, G. R., Michell, R. H. and Michelangeli, F. (1993) *Biochem. J.* **289**, 883–887
- 22 Galione, A., MacDougall, A., Busa, W. B., Willmott, N., Gillot, I. and Whitaker, M. (1993) *Science* **253**, 1143–1146
- 23 Tanaka, Y. and Tashjian, A. H. (1994) *J. Biol. Chem.* **269**, 11247–11253
- 24 Hellmich, M. R. and Strumwasser, F. (1991) *Cell Regul.* **2**, 193–202
- 25 Lee, H. C. and Aarhus, R. (1993) *Biochem. Biophys. Acta* **1164**, 68–74
- 26 Lee, H. C. (1991) *J. Biol. Chem.* **266**, 2276–2281
- 27 Walseth, T. F., Aarhus, R., Kerr, J. A. and Lee, H. C. (1993) *J. Biol. Chem.* **268**, 26686–26691
- 28 Galione, A., Lee, H. C. and Busa, W. B. (1991) *Science* **253**, 1143–1146
- 29 Lee, H. C. (1993) *J. Biol. Chem.* **268**, 293–299
- 30 Shen, S. S. and Buck, W. R. (1990) *Dev. Biol.* **140**, 272–280
- 31 Shen, S. S. (1989) *Biochem. Biophys. Res. Commun.* **161**, 1100–1108
- 32 Darszon, A., Lievano, A. and Beltran, C. (1996) *Curr. Topics Dev. Biol.* **34**, 117–165
- 33 Galione, A., White, A., Willmott, N., Turner, M., Potter, B. V. L. and Watson, S. P. (1993) *Nature (London)* **365**, 456–459
- 34 Genazzani, A. A., Mezna, M., Dickey, D. M., Michelangeli, F., Walseth, T. F. and Galione, A. (1997) *Br. J. Pharmacol.* **271**, 1489–1495
- 35 Brunder, D. G., Dettbarn, C. and Palade, P. (1988) *J. Biol. Chem.* **263**, 18785–18792
- 36 Favero, T., Zable, A. C. and Abramson, J. J. (1995) *J. Biol. Chem.* **270**, 25557–25563
- 37 Bootman, M. D., Taylor, C. W. and Berridge, M. J. (1992) *J. Biol. Chem.* **267**, 25113–25119
- 38 Abramson, J. J. and Salama, G. (1987) *J. Membr. Biol.* **33**, 241–248
- 39 Genazzani, A. A., Empson, R. M. and Galione, A. (1996) *J. Biol. Chem.* **271**, 11599–11602
- 40 Aarhus, R., Dickey, D. M., Graeff, R. M., Gee, K. R., Walseth, T. F. and Lee, H. C. (1996) *J. Biol. Chem.* **271**, 8513–8516
- 41 Genazzani, A. A., Mezna, M., Summerhill, R. J., Galione, A. and Michelangeli, F. (1997) *J. Biol. Chem.* **271**, 7669–7675
- 42 Poenie, M., Alderton, J., Tsien, R. and Steinhardt, R. A. (1985) *Nature (London)* **315**, 147–152
- 43 Kusunoki, S. and Yasumasu, I. (1976) *Biochim. Biophys. Acta* **68**, 881–885
- 44 Ciapa, B., Pesando, D., Wilding, M. and Whitaker, M. (1994) *Nature (London)* **368**, 875–878