Different modulatory effects of elevated cyclic AMP on cytosolic Ca^{2+} spikes induced by phenylephrine or vasopressin in single rat hepatocytes

Antonio SANCHEZ-BUENO,* Isabel MARRERO† and Peter H. COBBOLD‡

Department of Human Anatomy and Cell Biology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

We show here, by aequorin measurements in single isolated rat hepatocytes, that elevation of cyclic AMP, by dibutyryl cyclic AMP, forskolin or glucagon, has different effects on oscillations in cytosolic concentration of free Ca^{2+} ('free Ca') induced by phenylephrine or vasopressin. Elevated cyclic AMP does not itself induce free Ca oscillations, but enhances both the peak free Ca and the frequency of spikes induced by phenylephrine. In

contrast, elevated cyclic AMP has no effect on peak free Ca of vasopressin-induced spikes, but markedly prolongs the falling phase, with the result that spiking frequency (peak to peak) falls, although the period between spikes of resting free Ca is usually decreased. The data provide another example of receptor-specific information being retained in the oscillator mechanism, with implications for models of the hepatocyte calcium oscillator.

INTRODUCTION

Cyclic AMP and free Ca both act as metabotropic second messengers in liver cells. Interaction between the two second messengers has long been known [1]. In hepatocyte populations, elevated cyclic AMP has been shown to potentiate several actions of Ca^{2+} -mobilizing agonists, such as free Ca increases [2–5], Ca^{2+} influx or efflux [2,4,6–8], and inositol lipid turnover [3,9–11]. Elevated cyclic AMP may also, in the absence of added Ca^{2+} -mobilizing hormones, increase free Ca in hepatocyte populations [3–5,12–14] and in single guinea-pig hepatocytes within a population [15]; however, other groups have been unable to detect any effect of elevated cyclic AMP on free Ca levels in rat hepatocyte populations [16–18] and in single guinea-pig hepatocytes within a population [19].

At the level of the single isolated hepatocyte, Ca^{2+} -mobilizing agonists induce repetitive free Ca transients ('spikes'), whose frequency is modulated by agonist dose [20,21]. The time course of each spike is different for different agonists, with the falling phase being more prolonged in the order: phenylephrine, ADP, vasopressin, angiotensin II, endothelin-1, ATP [21–24]. We have previously shown [25] that elevation of cyclic AMP, by exogenous dibutyryl cyclic AMP or forskolin, enhances the frequency of spiking induced by the α_1 -adrenergic agonist phenylephrine, and also increases peak free Ca in each spike. Here we show that elevation of cyclic AMP by dibutyryl cyclic AMP, forskolin or glucagon have different effects on spikes induced by vasopressin compared with spikes induced by phenylephrine. Thus, in vasopressin-stimulated cells elevation of cyclic AMP markedly prolongs the falling phase of the spikes without altering peak free Ca, and spiking frequency usually does not rise. These data show that cyclic AMP-dependent mechanisms potentiate free Ca spiking in different ways, depending on the Ca^{2+} -mobilizing agonist, and provide further evidence for agonist-specified information being retained in the hepatocyte free Ca oscillator.

MATERIALS AND METHODS

Hepatocytes were isolated from fed male Wistar-strain rats (180–250 g) by perfusion with collagenase (Boehringer) as de-

scribed previously [20,21]. Procedures for microinjection of single hepatocytes, the experimental conditions and data collection have been described previously [20,21]. Forskolin (Sigma) was dissolved in ethanol and stored in small batches at -20°C . Glucagon (Sigma) was dissolved in 0.01 M HCl and stored at 4°C . Dibutyryl cyclic AMP (Sigma) was dissolved directly in the perfusion medium, Williams' Medium E (Flow Laboratories), which was gassed with CO_2 /air (1:19) at 37°C .

RESULTS

In confirmation of previous observations [25], neither forskolin nor dibutyryl cyclic AMP alone induced any free Ca changes in the hepatocyte (results not shown). As previously reported [25], forskolin at concentrations of 1–10 μM increased both the frequency and the peak free Ca of phenylephrine-induced spikes (Figures 1a and 1c). Broadly similar results were found in phenylephrine-exposed cells when dibutyryl cyclic AMP (1–10 μM) was used to elevate cyclic AMP ([25]; Figures 2a and 2c). These Figures are representative of 15 cells (forskolin) or 12 cells (dibutyryl cyclic AMP). In contrast, when vasopressin was the agonist, the effect on free Ca spikes of elevation of cyclic AMP, by either forskolin or dibutyryl cyclic AMP, was different from the effect on phenylephrine-induced spikes. Low concentrations of forskolin (1 μM ; Figure 1b) or dibutyryl cyclic AMP (1 μM ; Figure 2b) induced a prolongation of the falling phase of vasopressin-induced spikes, from 10–12 s to, sometimes, 2–3 min. Peak free Ca did not rise in any of the six hepatocytes treated with forskolin, and in only one of the five hepatocytes treated with dibutyryl cyclic AMP did peak free Ca rise, by about 200 nM. Higher concentrations of forskolin (5 μM ; Figure 1d) or dibutyryl cyclic AMP (10 μM ; Figure 2d), comparable with those that increase frequency and peak free Ca on phenylephrine-induced spikes (Figures 1c and 2c), produced a free Ca plateau on the falling phase of the vasopressin-induced spike (six cells with forskolin, and six with dibutyryl cyclic AMP). Hepatocytes exposed to sub-threshold concentrations of vasopressin could be induced to generate free Ca spikes by elevated cyclic AMP (Figure 3). These spikes had a falling phase that was prolonged

Abbreviations used: free Ca, cytosolic concentration of free Ca^{2+} ; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C.

* Present address: Centro de Investigaciones Biomédicas, CSIC, Arturo Duperier 4, 28029 Madrid, Spain.

† Present address: Departamento de Endocrinología Celular y Molecular, Universidad, Las Palmas de Gran Canaria, Islas Canarias, Spain 35080.

‡ To whom correspondence should be addressed.

Figure 1 Effect of low (1 μM ; panels a and b) and moderate (5 or 10 μM ; panels c and d) concentrations of forskolin on free Ca spikes induced by 1 μM phenylephrine (panels a and c) and 0.2 or 0.4 nM vasopressin (panels b and d)

Time constants in all Figures were 10 s for resting free Ca levels, and 1 s during spikes.

compared with normal vasopressin-induced spikes [21,24], whereas peak free Ca was the same as in normal spikes. These data are representative of four cells.

Glucagon alone usually had no effect on free Ca. Concentrations of glucagon of 0.1 nM up to 1 μM , over periods from 10 min up to 100 min, showed no changes in free Ca (six cells). Four other cells exposed to 0.1–50 nM glucagon each showed a single spike, after delays of approx. 3 min, over periods of exposure of 10 min (results not shown); no further spikes were induced when the glucagon concentration was increased. The effects of glucagon on free Ca spiking induced by phenylephrine or vasopressin were indistinguishable from the effects of dibutyryl

cyclic AMP or forskolin. Again, different effects on spikes induced by phenylephrine or vasopressin were discernible. Thus, when phenylephrine was the agonist, glucagon at physiological concentrations (0.1 nM; Figure 4a) changed the frequency and peak free Ca, similar to the effects of forskolin (Figures 1a and 1c) or dibutyryl cyclic AMP (Figures 2a and 2c). However, when vasopressin was the agonist, glucagon at the same concentration and in the same hepatocyte had no consistent effect on the peak free Ca of the spikes, but the falling phase was prolonged (Figure 4b), much as is seen with low concentrations of forskolin (Figure 1b) or dibutyryl cyclic AMP (Figure 2b).

The plateau of free Ca induced by vasopressin in the presence

Figure 2 Effect of a low dose (1 μM) of dibutyryl cyclic AMP (db-cAMP) on spikes induced by phenylephrine (0.5 μM ; panel a) or vasopressin (0.1 nM; panel b), and the effect of a moderate dose (10 μM) of dibutyryl cyclic AMP on spikes induced by 1 μM phenylephrine (panel c) or 0.4 nM vasopressin (panel d)

of moderate doses of cyclic AMP-elevating agents (e.g. 5 μM forskolin, Figure 1d; 10 μM dibutyryl cyclic AMP, Figure 2d) can be mimicked by phenylephrine in the presence of very high doses of forskolin (50 μM ; Figure 5a), dibutyryl cyclic AMP (100 μM ; Figure 5b) or glucagon (10 μM ; Figure 5c). The subsequent re-appearance of oscillations in forskolin-treated cells (Figure 5a) occurred in 3/6 cells; the remaining three cells showed responses similar to that in Figure 5(b), with a non-oscillatory plateau. Likewise, 1/3 cells respond to high dibutyryl cyclic AMP with a pattern reminiscent of Figure 5(a). The response to glucagon (Figure 5c) was representative of three cells.

DISCUSSION

Earlier studies on hepatocyte populations showed a potentiation by elevated cyclic AMP of free Ca responses induced by Ca^{2+} -mobilizing agonists such as phenylephrine or vasopressin [2–5]. In this study we report potentiatory responses of hepatocyte free Ca oscillations, induced by phenylephrine or vasopressin, to three different agents that will elevate cytoplasmic cyclic AMP; dibutyryl cyclic AMP, forskolin and glucagon. By themselves, none of these agents significantly raised free Ca. The occasional single-spike response to glucagon contrasts with a report [26] that sub-nanomolar concentrations induce generation of inositol

Figure 3 Forskolin ($10\ \mu\text{M}$) induces spiking in a cell exposed to a sub-threshold concentration of vasopressin ($50\ \text{pM}$), to induce spikes with prolonged tails compared with normal vasopressin-induced spikes

phosphates in hepatocytes. We suggest that reports of free Ca responses to elevated cyclic AMP in hepatocyte populations [3–5, 12–15] may represent a cyclic AMP-mediated potentiation of purinoreceptor activation by endogenous ATP or ADP released from injured cells. In our experimental system we use a single solitary cell superfused with medium that does not come into contact with any other cells. Our data are consistent with free Ca measurements made on hepatocyte populations, which showed no free Ca response to elevated cyclic AMP [16–19].

We were particularly intrigued to find that the potentiating effect of cyclic AMP on a hepatocyte's oscillatory free Ca response depends on the species of the Ca^{2+} -mobilizing agonist. Thus elevation of cyclic AMP by low or moderate concentrations of forskolin ($1\text{--}10\ \mu\text{M}$), dibutyryl cyclic AMP ($1\text{--}10\ \mu\text{M}$) or glucagon ($0.1\ \text{nM}$) increases both the frequency and the peak free Ca of phenylephrine-induced spikes (Figures 1a, 1c, 2a, 2c, 4a). In contrast, when vasopressin is the agonist there is no rise in peak free Ca, and the spiking frequency monitored peak-to-peak is decreased (Figures 1b, 2, 4b). But the period when free Ca is at resting levels, between the end of one spike's tail and the onset of the next spike, is usually markedly decreased. Indeed, a decrease in the inter-transient interval during which free Ca is at resting levels is, apart from the retention of very similar rates of rise of Ca in the spikes, the only parameter that cyclic AMP modifies similarly whichever agonist is used. Particularly striking is the observation that low levels of cyclic AMP prolong the falling phase of vasopressin-induced spikes (Figures 1b, 2b, 4b); no such effect is seen in phenylephrine-induced spikes. Somewhat higher elevation of cyclic AMP, induced by moderate concentrations of forskolin ($5\ \mu\text{M}$; Figure 1d) or dibutyryl cyclic AMP ($10\ \mu\text{M}$; Figure 2d), convert vasopressin-induced spikes into a sustained free Ca rise referred to as a 'plateau' (Figures 1d, 2d). High concentrations of forskolin ($50\ \mu\text{M}$; Figure 5a), dibutyryl cyclic AMP ($100\ \mu\text{M}$; Figure 5b) or glucagon ($10\ \mu\text{M}$; Figure 5c) are needed to produce a 'plateau'-type response in cells responding to phenylephrine.

The main question posed by these data is what mechanism can explain how the same cyclic-AMP-elevating protocols can exert different effects on free Ca spikes induced by phenylephrine or vasopressin? (Contrast Figures 1a with 1b, 2a with 2b, and 4a with 4b.) Several targets could be proposed whereby cyclic AMP,

Figure 4 Effect of glucagon ($0.1\ \text{nM}$) on spikes induced by $0.5\ \mu\text{M}$ phenylephrine (panel a) or $0.8\ \text{nM}$ vasopressin (panel b)

presumably acting through protein kinase A (PKA), could exert effects on free Ca. Agonist-mediated rises in $\text{Ins}(1,4,5)\text{P}_3$ levels are potentiated by cyclic AMP [3,9–11]. But raising hormone concentration also increases $\text{Ins}(1,4,5)\text{P}_3$ levels, but only affects spiking frequency, not spike shape [20,21]. Phosphorylation by PKA of phospholipase C (PLC) has been demonstrated *in vitro*, but without any effect on PLC activity [27]. If PKA were to act solely by modulating the activity of Ca^{2+} -release channels, such as the $\text{Ins}(1,4,5)\text{P}_3$ receptor, as proposed by Burgess et al. [19], we have difficulty in explaining the agonist-specificity of the effects of cyclic AMP described here, since presumably $\text{Ins}(1,4,5)\text{P}_3$ generated by different hormone receptors acts on the same pool of $\text{Ins}(1,4,5)\text{P}_3$ receptors. The superimposability of free Ca waves induced by several agonists in the same hepatocyte [28] makes spatial separation of $\text{Ins}(1,4,5)\text{P}_3$ actions according to which hormone receptors were activated improbable. Likewise there are difficulties in proposing that the effects of cyclic AMP on $\text{Ins}(1,4,5)\text{P}_3$ metabolism [3,9–11] are different according to which agonist induces PLC activity. Explanations for the agonist-specificity in the effects of elevated cyclic AMP on free Ca spikes can be envisaged through phosphorylation by PKA of hormone receptors. Receptor phosphorylation could result in higher affinity for agonist, as has been shown for adrenaline binding to hepatocytes [2], or greater efficiency of coupling of the receptor

Figure 5 Effect of high doses of cyclic-AMP-elevating agents [panel a, 50 μM forskolin; panel b, 100 μM dibutyl cyclic AMP (db-cAMP); panel c, 10 nM glucagon] on spikes induced by 0.5 or 1 μM phenylephrine

to G-proteins, and hence to a potentiation of free Ca spiking. Moreover, an interaction of cyclic AMP at hormone-receptor level could conceivably promote different effects, depending on the agonist. PKA phosphorylates *in vitro* the α_1 -adrenoreceptor [29], but the physiological role of this is not known. It has been also suggested that the vasopressin V_1 -receptor is phosphorylated by PKA [10,30]. However, the free Ca response induced by fluoroaluminate is also potentiated by cyclic AMP [3]. This suggests that PKA could interact at the level of the G-protein(s), and hence induce different responses to cyclic AMP depending on the agonist, provided that the different receptors are coupled to different G-proteins and provided that 'receptor-controlled' models of the hepatocyte Ca^{2+} oscillator pertain [22–24]. The plateau response to vasopressin at moderate elevations of cyclic AMP (Figures 1d, 2d) and to phenylephrine at high elevation of cyclic AMP (Figures 5a–5c) is reminiscent of the effect of inhibition of protein kinase C (PKC) [31]. One could envisage an antagonistic effect of PKA directed either at PKC itself or against PKC-induced phosphorylation of receptors or G-proteins. For instance, the α_1 -adrenoreceptor has both PKC and PKA consensus phosphorylation sites [29], which could conceivably explain the ability of high levels of cyclic AMP to mimic PKC inhibition (Figure 5). Preliminary data show that phorbol-ester activation of PKC will convert a plateau response to vasopressin into repeating spikes (but this might simply be a consequence of decreased agonist–receptor binding, as proposed for the effects of phorbol esters on spiking frequency in normal cells [32]).

We have previously shown [24], as have others [33], that phenylephrine-induced free Ca spikes are insensitive to ryanodine, which blocks the channel responsible for Ca^{2+} -induced Ca^{2+} release ('CICR') in muscle cells in the open configuration [34–36]. Phenylephrine-induced free Ca spikes presumably therefore do not involve CICR (eliminating 'Two Pool' models based on CICR, e.g. [37,38]) and are most probably mediated by the $\text{Ins}(1,4,5)\text{P}_3$ receptor. Although the possibility of pulsatile generation of $\text{Ins}(1,4,5)\text{P}_3$, as proposed in 'receptor-controlled' models [21–24], and the concept of 'increment detection' of $\text{Ins}(1,4,5)\text{P}_3$ pulses [39], have not been eliminated, the complex dynamic behaviour of the $\text{Ins}(1,4,5)\text{P}_3$ receptor may permit pulsatile Ca release without $\text{Ins}(1,4,5)\text{P}_3$ levels oscillating. Features of $\text{Ins}(1,4,5)\text{P}_3$ -receptor behaviour that could promote sudden Ca^{2+} release at more or less constant $\text{Ins}(1,4,5)\text{P}_3$ levels are: co-operativity of $\text{Ins}(1,4,5)\text{P}_3$ action on Ca^{2+} release [40], multiple binding sites for $\text{Ins}(1,4,5)\text{P}_3$ on its receptor [41], a dependence of $\text{Ins}(1,4,5)\text{P}_3$ -sensitivity to luminal Ca^{2+} content [42], and positive feedback from low levels, approaching ~ 400 nM, of free Ca [43–45]. A rapid switch-off of the Ca^{2+} conductance of the $\text{Ins}(1,4,5)\text{P}_3$ receptor could occur in a few seconds [46] and be triggered by negative feedback from levels of free Ca of 700 nM [43–46] and, maybe, by luminal Ca^{2+} depletion. In this way both the rapid rise and fall of free Ca in each spike would reside, essentially, in the properties of the $\text{Ins}(1,4,5)\text{P}_3$ receptor itself. Phosphorylation of both brain and platelet InsP_3 receptor by PKA decreased $\text{Ins}(1,4,5)\text{P}_3$ -induced Ca^{2+} release [47,48]. In hepatocytes, however, Burgess et al. [8,19] describe a potentiation by elevated cyclic AMP of the $\text{Ins}(1,4,5)\text{P}_3$ -releasable pool, and which was measurable in permeabilized cells presented with cyclic AMP and the catalytic subunit of PKA [19], but as yet we do not know which parameter of spiking will be modified by this. Cyclic AMP-mediated enhancement of $\text{Ins}(1,4,5)\text{P}_3$ generation [3,9–11], which would be predicted to act like an increase in agonist dose, may underlie the cyclic AMP-induced increase in spiking frequency.

The mechanism proposed above for an $\text{Ins}(1,4,5)\text{P}_3$ -receptor-

centred spiking mechanism should also of course apply to vasopressin-mediated spiking. Indeed, the decrease in the inter-transient interval at which free Ca is at rest may have a similar cause to the enhancement of frequency of phenylephrine spiking, particularly if free Ca has to return to resting levels before the negative feedback of free Ca on to the $\text{Ins}(1,4,5)\text{P}_3$ receptor [43–45] is removed. The complication arises when we consider that with vasopressin there is no rise in peak free Ca and, especially, the enormous prolongation of the falling phase of the vasopressin-induced spikes. However, we show in the following paper [49] that vasopressin-induced free Ca spiking is sensitive to ryanodine, in contrast with the ryanodine-insensitive spiking induced by phenylephrine [24,33], and that ryanodine can truncate the cyclic-AMP-prolonged tails of vasopressin-induced spikes. So there may be a difference in Ca^{2+} pooling between phenylephrine- and vasopressin-treated cells which may be central to understanding the agonist-specificity of the cyclic AMP effects described here.

We are grateful for funding by the Basque Government (A.S.-B), the Canary Island Government (I.M.) and The Wellcome Trust.

REFERENCES

- Berridge, M. J. (1975) *Adv. Cyclic Nucleotide Res.* **6**, 1–98
- Morgan, N. G., Charest, R., Blackmore, P. F. and Exton, J. H. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4208–4212
- Blackmore, P. F. and Exton, J. H. (1986) *J. Biol. Chem.* **261**, 11056–11063
- Combettes, L., Berthon, B., Binet, A. and Claret, M. (1986) *Biochem. J.* **237**, 675–683
- Connely, P. A., Botelho, L. H. P., Sick, R. B. and Garrison, J. C. (1987) *J. Biol. Chem.* **262**, 4324–4332
- Mauger, J.-P., Poggioli, J. and Claret, M. (1985) *J. Biol. Chem.* **260**, 11635–11642
- Poggioli, J., Mauger, J.-P. and Claret, N. (1986) *Biochem. J.* **235**, 663–669
- Burgess, G. M., Dooley, R. K., McKinney, J. S., Nanberg, E. and Putney, J. W. (1986) *Mol. Pharmacol.* **30**, 315–320
- Whipps, D. E., Armston, A. E., Pryor, H. J. and Halestrap, A. P. (1987) *Biochem. J.* **241**, 835–845
- Pittner, R. A. and Fain, J. N. (1989) *Biochem. J.* **257**, 455–460
- Pittner, R. A. and Fain, J. N. (1990) *Biochim. Biophys. Acta* **1043**, 211–217
- Charest, R., Blackmore, P. F., Berthon, B. and Exton, J. H. (1983) *J. Biol. Chem.* **258**, 8769–8773
- Sistare, F. D., Picking, R. A. and Haynes, R. C., Jr. (1985) *J. Biol. Chem.* **260**, 12744–12747
- Staddon, J. M. and Hansford, R. G. (1989) *Biochem. J.* **179**, 47–52
- Capiod, T., Noel, J., Combettes, L. and Claret, M. (1991) *Biochem. J.* **275**, 277–280
- Murphy, E., Coll, K., Rich, T. L. and Williamson, J. R. (1980) *J. Biol. Chem.* **255**, 6600–6608
- Williamson, J. R., Cooper, R. H. and Hoeck, J. B. (1981) *Biochim. Biophys. Acta* **639**, 243–295
- Studer, R. K., Snowdowne, K. W. and Borle, A. B. (1984) *J. Biol. Chem.* **259**, 3596–3604
- Burgess, G. M., Bird, G. St. J., Obie, J. F. and Putney, J. W., Jr. (1991) *J. Biol. Chem.* **266**, 4772–4781
- Woods, N. M., Cuthbertson, K. S. R. and Cobbold, P. H. (1986) *Nature (London)* **319**, 600–602
- Woods, N. M., Cuthbertson, K. S. R. and Cobbold, P. H. (1987) *Cell Calcium* **8**, 79–100
- Sanchez-Bueno, A., Dixon, C. J., Woods, N. M., Cuthbertson, K. S. R. and Cobbold, P. H. (1990) *Adv. Second Messenger Phosphoprotein Res.* **24**, 115–121
- Cobbold, P. H., Dixon, C. J., Sanchez-Bueno, A., Woods, N. M., Daly, M. and Cuthbertson, K. S. R. (1990) in *Transmembrane Signalling: Intracellular Messengers and Implications for Drug Development* (Nahorski, S. R., ed.), pp. 185–206, Wiley, Chichester
- Cobbold, P. H., Sanchez-Bueno, A. and Dixon, C. J. (1991) *Cell Calcium* **12**, 87–96
- Schöfl, C., Sanchez-Bueno, A., Brabant, G., Cobbold, P. H. and Cuthbertson, K. S. R. (1991) *Biochem. J.* **273**, 799–802
- Wakelam, M. J. O., Murphy, G. J., Hraby, V. J. and Houslay, M. D. (1986) *Nature (London)* **323**, 68–71
- Kim, U.-H., Kim, J. W. and Rhee, S. G. (1989) *J. Biol. Chem.* **264**, 20167–20170
- Rooney, T. A., Sass, E. J. and Thomas, A. P. (1990) *J. Biol. Chem.* **265**, 10792–10796
- Bouvier, M., Leeb-Lunberg, L. M. F., Benovic, J. L., Caron, M. G. and Lefkowitz, R. J. (1987) *J. Biol. Chem.* **262**, 3106–3113
- Guillon, G., Gallo-Payet, N., Balestre, M. N. and Lombard, C. (1988) *Biochem. J.* **253**, 765–775
- Woods, N. M., Cuthbertson, K. S. R. and Cobbold, P. H. (1987) *Biochem. J.* **246**, 619–623
- Sanchez-Bueno, A., Dixon, C. J., Woods, N. M., Cuthbertson, K. S. R. and Cobbold, P. H. (1990) *Biochem. J.* **268**, 627–632
- Somogyi, R. and Stucki, J. W. (1990) *J. Biol. Chem.* **266**, 11068–11077
- Fleischer, S., Ogunbunmi, E. M., Dixon, M. C. and Fleer, E. A. M. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7256–7259
- Iino, M., Kobayashi, T. and Endo, M. (1988) *Biochem. Biophys. Res. Commun.* **152**, 417–422
- Ma, J., Fill, M., Knudson, C. M., Campbell, K. P. and Coronado, R. (1988) *Science* **242**, 99–102
- Berridge, M. J. (1990) *J. Biol. Chem.* **265**, 9583–9586
- Berridge, M. J. and Galione, A. (1988) *FASEB J.* **2**, 3074–3082
- Meyer, T. and Stryer, L. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3841–3845
- Meyer, T., Holowka, D. and Stryer, L. (1988) *Science* **240**, 653–655
- Meyer, T., Wensel, T. and Stryer, L. (1990) *Biochemistry* **29**, 32–37
- Missiaen, L., Taylor, C. W. and Berridge, M. J. (1991) *Nature (London)* **352**, 241–244
- Iino, M. (1990) *J. Gen. Physiol.* **95**, 1103–1122
- Bezprozvanny, I., Watras, J. and Ehrlich, E. E. (1991) *Nature (London)* **351**, 751–754
- Finch, E. A., Turner, T. J. and Goldin, S. M. (1991) *Science* **252**, 443–446
- Pietri, F., Hilly, M. and Mauger, J. P. (1990) *J. Biol. Chem.* **265**, 17478–17485
- Snyder, S. H. and Suppatapone, S. (1989) *Cell Calcium* **10**, 337–342
- Tohmatsu, T., Nishida, A., Nagao, S., Nakashima, S. and Nozawa, Y. (1989) *Biochim. Biophys. Acta* **1013**, 190–193
- Sanchez-Bueno, A. and Cobbold, P. H. (1993) *Biochem. J.* **291**, 169–172