Biochem. J. (1989) 260, 771–775 (Printed in Great Britain)

Mode of activation of bovine brain inositol 1,4,5-trisphosphate 3-kinase by calmodulin and calcium

Guodong LI,* Michelle COMTE,† Claes B. WOLLHEIM* and Jos A. COX†‡
†Department of Biochemistry and *Institut de Biochimie Clinique, University of Geneva, 1211 Geneva 4, Switzerland

The effect of Ca^{2+} and calmodulin (CaM) on the activation of purified bovine brain Ins(1,4,5)P_3 kinase was quantified and interpreted according to the model of sequential equilibria generally used for other calmodulin-stimulated systems. Two main conclusions can be drawn. (i) $CaM \cdot Ca_3$ and $CaM \cdot Ca_4$ together are the biologically active species *in vitro*, as is the case for the great majority of other calmodulin targets. (ii) These species bind in a non-co-operative way to the enzyme with an affinity constant of 8.23×10^9 M^{-1}, i.e. approx 10-fold higher than for most calmodulin-activated target enzymes. The dose–response curve of the activation of Ins(1,4,5)P_3 kinase by calmodulin is not significantly impaired by melittin and trifluoperazine, whereas under very similar assay conditions the half-maximal activation of bovine brain cyclic AMP phosphodiesterase requires over 30–50-fold higher concentrations of CaM when 1 μM melittin or 20 μM-trifluoperazine is present in the assay medium. Similarly, 1 μM of the anti-calmodulin peptides seminalplasmin and gramicidin S, as well as 20 μM of *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulphonamide (W7), do not inhibit the activation process. These data suggest that binding and activation of Ins(1,4,5)P_3 kinase require surface sites of calmodulin which are different from those involved in the binding of most other target enzymes or of model peptides.

INTRODUCTION

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3], generated by receptor-regulated hydrolysis of phosphatidylinositol 4,5-bisphosphate, is a second messenger for the mobilization of Ca^{2+} from intracellular stores (Berridge & Irvine, 1984). It can be inactivated by dephosphorylation to D-*myo*-inositol 1,4-bisphosphate (Storey *et al.*, 1984) or by phosphorylation to Ins(1,3,4,5)P_4 (Irvine *et al.*, 1986). Ins(1,4,5)P_3 kinase, the enzyme involved in the latter conversion, is predominantly soluble and displays a high specificity and affinity for its substrates, Ins(1,4,5)P_3 and ATP. Biden & Wollheim (1986) have reported that the enzyme from RINm5F insulinoma cells is regulated by micromolar concentrations of Ca^{2+}. Subsequently it was established that Ins(1,4,5)P_3 kinase originating from these cells (Biden *et al.*, 1987), from aortic smooth muscle (Yamaguchi *et al.*, 1987), from macrophages (Kimura *et al.*, 1987), from bovine brain (Ryu *et al.*, 1987), and the membrane-bound form of the enzyme from turkey erythrocytes (Morris *et al.*, 1987) are activated 3–5-fold by Ca^{2+}–CaM. More recently a method based on the Ca^{2+}-dependent and reversible interaction of Ins(1,4,5)P_3 kinase with CaM–agarose was developed (Johanson *et al.*, 1988) for the purification of the enzyme from rat brain. This purified enzyme was, however, poorly activated by CaM (1.5–2-fold), as were also the enzymes in the crude extracts of rat and bovine brain (Takazawa *et al.*, 1988). Our recent attempts to purify Ins(1,4,5)P_3 kinase from bovine brain yielded preparations which are activated up to 25-fold by Ca^{2+}–CaM (M. Comte, G. Li, C. B. Wollheim & J. A. Cox, unpublished work).

The purpose of the present work was to provide a quantitative description of the activation of Ins(1,4,5)P_3 kinase by CaM and Ca^{2+} according to a model that has been used for many other target enzymes of CaM (for review, see Cox, 1984). This study includes the comparison of the activation parameters of different Ca^{2+}–CaM targets in order to evaluate the physiological relevance of this particular regulatory pathway. We also have evaluated the inhibitory potential of the well-known CaM antagonists melittin (Comte *et al.*, 1983), seminalplasmin (Comte *et al.*, 1983), trifluoperazine (Levin & Weiss, 1976) and *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulphonamide (W7) (Hidaka & Tanaka, 1983) in the activation of Ins(1,4,5)P_3 kinase.

EXPERIMENTAL

Ins(1,4,5)P_3 kinase was partially (12000–60000-fold) purified from bovine brain to a specific activity of 2–10 μmol/min per mg of protein by the following steps: anion and cation exchange, chromatography on phosphocellulose P11 with phytic acid gradient eluent, and on Affi-Blue Gel with salt-gradient elution, and affinity chromatography on MINI LEAK (from Bio-Carb AB, Lund, Sweden) immobilized CaM (M. Comte, G. Li, C. B. Wollheim & J. A. Cox, unpublished work). Bovine brain calmodulin was purified as described by Gopalakrishna & Anderson (1982) and bovine brain cyclic AMP phosphodiesterase as described previously (Cox *et al.*, 1981). Bee venom melittin, bull seminalplasmin and gramicidin S were purified as described by Maulet & Cox (1983), Comte *et al.* (1986) and Cox *et al.* (1987) respectively. The trifluoperazine and W7 (both from Sigma Chemical Co., St Louis, MO, U.S.A.) stock solutions were in water. The Ins(1,4,5)P_3 kinase assays were carried out as previously described (Biden &

Abbreviations used: CaM, calmodulin; W7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulphonamide.

‡ To whom reprint requests should be addressed at: Department of Biochemistry, 30 quai Ernest-Ansermet, 1211 Geneva 4, Switzerland.

Wollheim, 1986). The incubation mixtures contained 10 mM-Hepes, pH 7.0, 110 mM-KCl, 10 mM-NaCl, 7 mM-$MgSO_4$, 5 mM-ATP, 5 mM-cysteine, 5 mM-EGTA, 0.5 μM-Ins(1,4,5)P_3, 0.1 μCi of [2-^{3}H]Ins(1,4,5)P_3/ml, 0.1 mg of bovine serum albumin/ml and 3 mM-2,3-bisphosphoglycerate in order to inhibit maximally the inositolphosphate phosphatases (Berridge *et al.*, 1983). Free Ca^{2+} concentrations were determined in 5 mM-EGTA-buffered media with a Ca^{2+}-specific electrode (Prentki *et al.*, 1983). Ins(1,3,4,5)P_4 was separated by anion exchange on Dowex 1-X8 columns (Berridge *et al.*, 1983). The distribution of the CaM·Ca_n species as a function of free [Ca^{2+}] was calculated as previously described (Cox *et al.*, 1981), by using the following stoichiometric Ca^{2+}-binding constants: $K_1 = 1.16 \times 10^5$ M^{-1}, $K_2 = 2.65 \times 10^5$ M^{-1}, $K_3 = 8.33 \times 10^4$ M^{-1} and $K_4 = 1.91 \times 10^4$ M^{-1} (Burger *et al.*, 1984). The cyclic AMP phosphodiesterase assay was carried out at 30 °C as described by Boudreau & Drummond (1975). For both Ins(1,4,5)P_3 kinase and cyclic AMP phosphodiesterase, the inhibition experiments with the CaM antagonists were carried out as described previously (Comte *et al.*, 1983). The Scatchard analysis, which was chosen to quantify the enzyme-activation data, consists in a linearization of the CaM-dependent activation according to the following equation:

$$(\text{Act.}_i - \text{Act.}_{basal})/[\text{CaM}_{total}]_i = K_a(\text{Act.}_{max} - \text{Act.}_{basal}) - K_a(\text{Act.}_i - \text{Act.}_{basal})$$

where Act._{basal}, Act._i and Act._{max} are the enzyme activities in the absence (basal) and presence of sub-saturating (i) or saturating (max.) concentrations of CaM, and K_a is the association constant of CaM for Ins(1,4,5)P_3 kinase. Under the experimental conditions described above, at most 30% of Ins(1,4,5)P_3 was converted and the enzyme activities are linearly related to the production of [2-^{3}H]Ins(1,3,4,5)P_4.

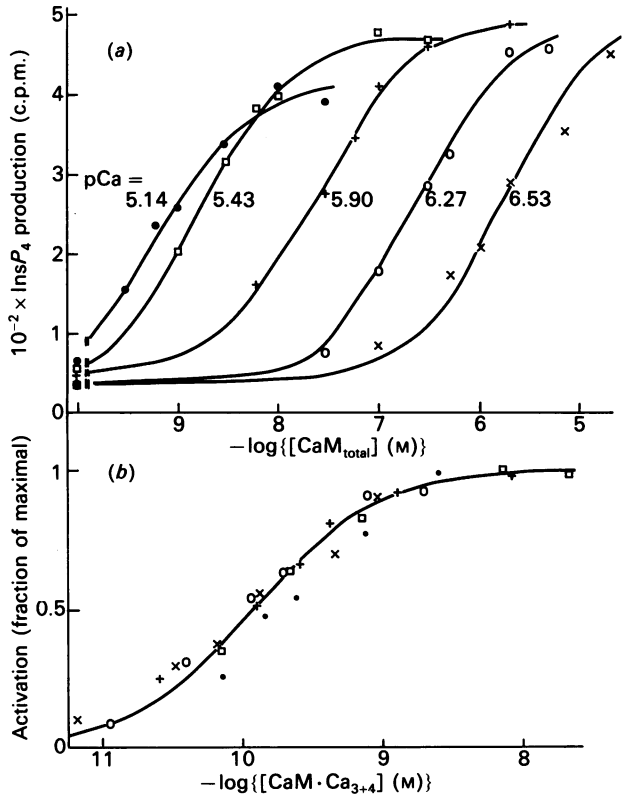

Fig. 1. Activation of Ins(1,4,5)P_3 kinase as a function of the total CaM concentration at five selected concentrations of free Ca^{2+} (*a*) and as a function of CaM·$Ca_{n \geq 3}$ (*b*)

The continuous lines in (*a*) are the activation curves calculated by using the K_m and $V_{max.}$ obtained after linearization of the experimental data.

RESULTS

A prerequisite for a quantitative analysis of the activation of the enzyme by Ca^{2+} and CaM is that CaM does not significantly change the Michaelis constant of the enzyme for Ins(1,4,5)P_3 (Cox *et al.*, 1982). The K_m for ATP is 192 μM (mean of two determinations) in the presence of 10 μM-CaM and 10 μM-Ca^{2+}. The K_m of the enzyme for Ins(1,4,5)P_3 is 0.52 ± 0.08 μM ($n = 3$) in the presence of 5 mM-EGTA, 0.51 ± 0.02 ($n = 3$) in the presence of 10 μM-Ca^{2+} and 0.50 ± 0.04 ($n = 3$) in the presence of 10 μM-Ca^{2+} + 10 μM-CaM (Fig. 1). CaM increases $V_{max.}$ by a factor of 4–25, depending on the enzyme preparation. Johanson *et al.* (1988) reported that CaM causes a 2-fold increase in both the K_m and $V_{max.}$ for Ins(1,4,5)P_3 in their preparation. The discrepancy may result from the different purification procedures or from differences between the enzyme from rat and bovine brain. Ca^{2+} alone does not modify significantly the kinetic parameters of Ins(1,4,5)P_3 kinase up to 30 μM free cation (results not shown). This is at variance with the strong inhibitory effect of Ca^{2+} at concentrations above 2 μM described for the bovine brain enzyme by Ryu *et al.* (1987).

The quantitative analysis of the activation of Ins(1,4,5)P_3 kinase by CaM + Ca^{2+} is based on the following detailed reaction mechanism:

$$\text{CaM} + \text{Ca}^{2+} \overset{K_1}{\longleftrightarrow} \text{CaM} \cdot \text{Ca}$$

$$\text{CaM} \cdot \text{Ca} + \text{Ca}^{2+} \overset{K_2}{\longleftrightarrow} \text{CaM} \cdot \text{Ca}_2$$

$$\text{CaM} \cdot \text{Ca}_{n-1} + \text{Ca}^{2+} \overset{K_n}{\longleftrightarrow} \text{CaM}^*\text{Ca}_n$$

$$\text{Enzyme} + \text{CaM}^*\text{Ca}_n \overset{K_a}{\longleftrightarrow} \text{Enzyme}^*\text{CaM}^*\text{Ca}_n$$

where K_1, K_2 and K_n are the respective stoichiometric binding constants of Ca^{2+} for CaM, CaM*Ca_n is the species that is capable of activating the enzyme and K_a is the association constant of the activating CaM species with Ins(1,4,5)P_3 kinase. In order to define the parameters K_a and n, the CaM-dependence of Ins(1,4,5)P_3 kinase was studied at five selected concentrations of free Ca^{2+} from 0.3 to 7 μM (Fig. 1*a*). A Scatchard analysis of each of the activation curves (Table 1) indicates that, except for the experiment at the highest free Ca^{2+} concentration, the same maximal activation is obtained, but that higher concentrations of CaM are required to induce half-maximal activation at lower concentrations of free Ca^{2+}. As with most CaM-dependent enzymes (Cox, 1984), the activation of Ins(1,4,5)P_3 kinase by CaM does not show positive or negative co-operativity. At half-maximal activation the concentration of the activating CaM

Table 1. Activation parameters of Ins(1,4,5)P_3 kinase by CaM as obtained by Scatchard analysis of the data of Fig. 1(*a*) and concentrations of the CaM·Ca_{3+4} species at half-maximal activation

Free [Ca^{2+}] (μM)	Maximal activity* (c.p.m.)	Apparent K_d† (nM)	[CaM·Ca_{3+4}] (nM)
2.97×10^{-7}	450	1670	0.109
5.42×10^{-7}	460	264	0.102
1.25×10^{-6}	450	28.7	0.123
3.73×10^{-6}	415	1.80	0.129
7.24×10^{-6}	352	0.79	0.155

* Scatchard analyses were performed after subtraction of the basal activities (activity in the absence of CaM).
† $K_d = 1/K_a$; K_a is defined in the text.

species is constant ($K_a = 1/[CaM^*Ca_n]$) [the accuracy of our analysis rests on the assumption that the free and total concentrations of CaM in the assay are not significantly different; this assumption is very likely, given the high degree of purification of Ins(1,4,5)P_3 kinase and the fact that in the assay its concentration does not exceed 10^{-11} M, as calculated on the basis of the specific activity reported by Johanson *et al.* (1988)] and the same in the five activation curves of Fig. 1(*a*). Table 1 shows that this requirement is met provided that $n \geqslant 3$, suggesting that CaM·Ca_3 and CaM·Ca_4 are the biologically activating species. This is fully confirmed when activation of Ins(1,4,5)P_3 kinase was plotted as a function of the sum of concentrations of CaM·Ca_3 and CaM·Ca_4 (Fig. 1*b*): one single non-co-operative isotherm is generated, with a K_a of 8.23 ($\pm$0.17) $\times 10^9$ M^{-1} (four independent experiments on different enzyme preparations).

The effect of the two well-known CaM antagonists melittin and trifluoperazine on the activation of Ins(1,4,5)P_3 kinase by CaM at 1.47 μM free Ca^{2+} is shown in Fig. 2. A Scatchard analysis of the three activation curves yielded apparent K_d values of 31 (control), 28 (+melittin) and 29 nM (+trifluoperazine) respectively. None of the CaM antagonists affects the basal activity, whereas only trifluoperazine has a small inhibitory effect on the maximal activation of the enzyme. Under very similar assay conditions and at the same drug concentrations, the CaM dose–response curves of phosphodiesterase are shifted to 33- and 50-fold higher CaM concentrations in the presence of melittin and trifluoperazine respectively (results not shown). Clearly these two classical CaM antagonists act very differently on the two CaM-regulated enzymes studied here. Table 2 further shows that other CaM antagonists such as seminalplasmin (Comte *et al.*, 1986), gramicidin S (Cox *et al.*, 1987) and W7 (Hidaka & Tanaka, 1983) have little or no effect on the activation of Ins(1,4,5)P_3 kinase by CaM, even in the sensitive part of the activation curve. It should be noted that very high concentrations of trifluoperazine (100 μM) inhibit the CaM activation, but not the basal activity of the enzyme (results not shown); it is, however, not clear whether they affect the apparent K_d or the extent of maximal activation.

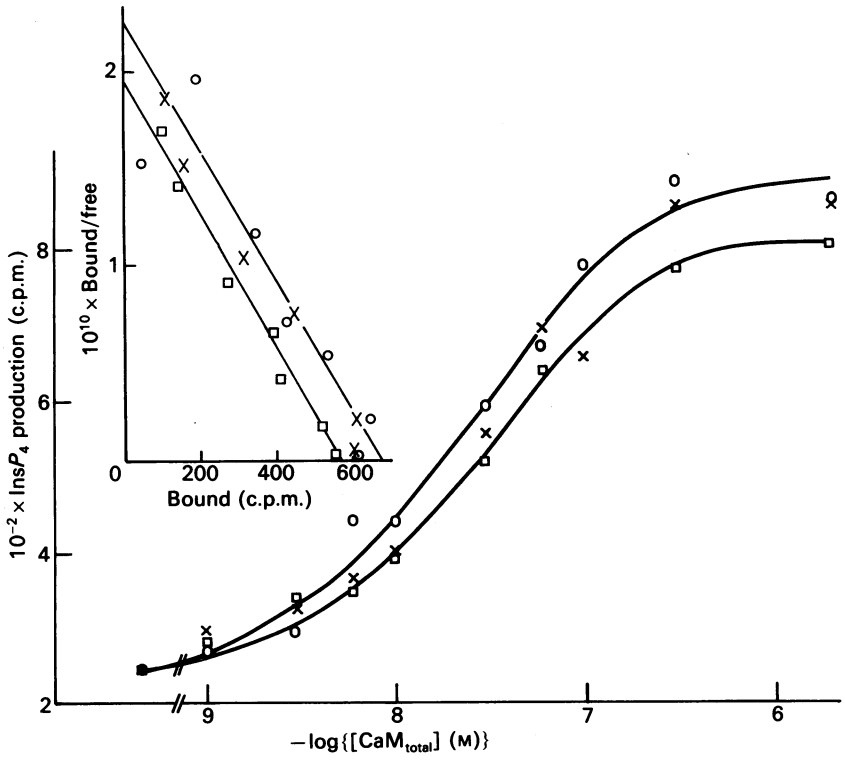

Fig. 2. Activation of Ins(1,4,5)P_3 kinase by CaM at 1.47 μM free Ca^{2+} in the absence (○) and in the presence of either 1 μM-melittin (×) or 20 μM-trifluoperazine (□)

The inset represents the Scatchard plot of the same data (some of the points at the two lowest CaM concentrations have not been included).

Table 2. Influence of CaM antagonists on the activation of Ins(1,4,5)P_3 kinase by 0.1 μM-CaM at 1.47 μM free Ca^{2+}

Values are means ± S.E.M. for three experiments.

		Activity (c.p.m.)
−CaM	−	108 ± 1.4
+CaM	−	429 ± 25.2*
+CaM	+1 μM-melittin	435 ± 11.6
+CaM	+1 μM-seminalplasmin	401 ± 15.8
+CaM	+1 μM-gramicidin S	422 ± 3.9
+CaM	+20 μM-W7	434 ± 29.4
+CaM	+20 μM-trifluoperazine	370 ± 18.8

* At 0.1 μM-CaM and 1.47 μM free Ca^{2+}, this corresponds to 75% of maximal activation by CaM.

DISCUSSION

The quantitative analysis of Ins(1,4,5)P_3 kinase activation by Ca^{2+}-CaM presented here is based on the premises that, at a given degree of saturation of CaM by Ca^{2+}, the resulting species is capable of high-affinity interaction with the enzyme, leading to maximal activation of the latter. Owing to the principle of 'linked functions' (Wyman, 1964), also called principle of 'free energy coupling' (Weber, 1975), the affinities of the individual components in the ternary complex will change, but this does not compromise the approach used here, which describes only the stoichiometry and affinity of the free $CaM \cdot Ca_n$ species which is in direct equilibrium with the activated enzyme. A more complete thermodynamic description of the interactions between Ins(1,4,5)P_3 kinase, CaM and Ca^{2+}, as has been done for smooth-muscle myosin light-chain kinase (Mamar-Bachi & Cox, 1988), is not yet feasible for lack of knowledge of the affinity of metal-free CaM for the enzyme and of the affinities of Ca^{2+} for enzyme-bound CaM.

Our analysis revealed that, as with other enzymes assayed in our laboratory, e.g. bovine brain cyclic nucleotidase phosphodiesterase (Cox *et al.*, 1981), bovine cerebellar adenylate cyclase (Malnoe *et al.*, 1982), the Ca-pump ATPase of human erythrocytes (Cox *et al.*, 1982), fast-skeletal-muscle phosphorylase *b* kinase (Burger *et al.*, 1983) and smooth-muscle myosin light-chain kinase (Mamar-Bachi & Cox, 1988), $CaM \cdot Ca_{n \geq 3}$ is the activating species. However, the affinity of Ins(1,4,5)P_3 kinase for the active species is approx 10-fold higher than that of the above-mentioned enzymes. With its affinity of approx. 10^{10} M^{-1} for CaM, Ins(1,4,5)P_3 kinase is, together with calcineurin (Hubbard & Klee, 1987), the strongest CaM ligand in brain. It should be noted that heart cyclic nucleotide phosphodiesterase, in contrast with the isoforms in brain, is also half-maximally activated at 0.1 nM free CaM (Hansen & Beavo, 1986). In the latter report it was argued that the difference in the CaM activation constants of the heart and brain enzymes evolved in relation to their respective cytosolic CaM contents. The higher activation of Ins(1,4,5)P_3 kinase by CaM in brain might reflect a reinforcement pathway of the Ca^{2+} signal: the primary agonist-induced mobilization of microsomal Ca^{2+} by Ins(1,4,5)P_3 leads to an increase in the concentration of $CaM \cdot Ca_{n \geq 3}$, which preferentially (hence primordially) activates Ins(1,4,5)P_3 kinase. It has been suggested that the resulting increase in Ins(1,3,4,5)P_4 would lead to a further increase in cytosolic Ca^{2+} (Irvine, 1987) and consequently to higher concentrations of $CaM \cdot Ca_{n \geq 3}$. Consequently a great number of CaM-dependent enzymes will be activated.

Surprisingly, neither 1 μM-melittin, which binds to CaM in a 1:1 complex with an affinity of 10^9 M^{-1} (Comte *et al.*, 1983), nor 20 μM-trifluoperazine, which forms a 2:1 complex with CaM with an affinity of approx. 10^6 M^{-1} for each drug molecule (Levin & Weiss, 1978), noticeably affects the half-maximal activation of Ins(1,4,5)P_3 kinase by CaM. This is very puzzling, since their inhibitory effect has been established in the case of many CaM-regulated targets. The lack of inhibition cannot be correlated directly to the approx. 10-fold higher affinity of Ins(1,4,5)P_3 kinase for CaM, since for phosphodiesterase even 60 nM-melittin shifts the CaM dose–response curve to the right by one order of magnitude (Comte *et al.*, 1983). Other CaM antagonists, such as seminalplasmin (Comte *et al.*, 1986) and W7 (Hidaka & Tanaka, 1983), also do not significantly inhibit Ins(1,4,5)P_3 kinase at doses which strongly affect other CaM-regulated enzymes. Previously (Biden *et al.*, 1987) we observed that the CaM-activated Ins(1,4,5)P_3 kinase from insulin-secreting RINm5F cells is inhibited by W7, but at 10–20-fold higher doses than reported for cyclic AMP phosphodiesterase and myosin light-chain kinase (Hidaka & Tanaka, 1983). With their high affinity for CaM, trifluoperazine and especially melittin must, at the concentrations used in the assay, sequester virtually all free $CaM \cdot Ca_n$. It thus seems likely that the binding site(s) for melittin and trifluoperazine on the surface of CaM, which is believed to correspond to the site involved in the activation of most CaM-regulated enzymes, is different from the site(s) involved in the interaction with and activation of Ins(1,4,5)P_3 kinase. In this respect it is noteworthy that gramicidin S, although it forms a 2:1 complex of high affinity with CaM (Cox *et al.*, 1987), does not efficiently inhibit CaM-activated cyclic AMP phosphodiesterase. For reasons enumerated in that study (Cox *et al.*, 1987), we assumed that a ternary enzyme–CaM–antagonist complex is formed, which is as active as the enzyme–CaM complex. Also in the present study the data suggest that a similar phenomenon occurs, i.e. formation of enzymically active ternary complexes between Ins(1,4,5)P_3 kinase, CaM and melittin (or trifluoperazine). An important practical consequence of these findings is that trifluoperazine and W7, frequently used as anti-CaM drugs in cell studies, do not abolish all the biological actions of CaM.

This work was supported by Swiss National Science Foundation Grants 3.214-085 and 3.577-087.

REFERENCES

Berridge, M. J. & Irvine, R. F. (1984) Nature (London) **312**, 315–321

Berridge, M. J., Dawson, R. M. C., Downes, C.P., Heslop, J. P. & Irvine, R. F. (1983) Biochem. J. **212**, 473–482

Biden, T. J. & Wollheim, C. B. (1986) J. Biol. Chem. **261**, 11931–11934

Biden, T. J., Comte, M., Cox, J. A. & Wollheim, C. B. (1987) J. Biol. Chem. **262**, 9437–9440

Boudreau, R. J. & Drummond, G. I. (1975) Anal. Biochem. **63**, 388–399
Burger, D., Stein, E. A. & Cox, J. A. (1983) J. Biol. Chem. **258**, 14733–14739
Burger, D., Cox, J. A., Comte, M. & Stein, E. A. (1984) Biochemistry **23**, 1966–1971
Comte, M., Maulet, Y. & Cox, J. A. (1983) Biochem. J. **209**, 269–272
Comte, M., Malnoë, A. & Cox, J. A. (1986) Biochem. J. **240**, 567–573
Cox, J. A. (1984) Fed. Proc. Fed. Am. Soc. Exp. Biol. **43**, 3000–3004
Cox, J. A., Malnoë, A. & Stein, E. A. (1981) J. Biol. Chem. **256**, 3218–3222
Cox, J. A., Comte, M. & Stein, E. A. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 4265–4269
Cox, J. A., Milos, M. & Comte, M. (1987) Biochem. J. **246**, 495–502
Gopalakrishna, R. & Anderson, W. B. (1982) Biochem. Biophys. Res. Commun. **104**, 830–836
Hansen, R. S. & Beavo, J. A. (1986) J. Biol. Chem. **261**, 14636–14645
Hidaka, H. & Tanaka, T. (1983) Methods Enzymol. **102**, 185–194
Hubbard, M. J. & Klee, C. B. (1987) J. Biol. Chem. **262**, 15062–15070
Irvine, R. F. (1987) Nature (London) **328**, 386
Irvine, R. F., Letcher, A. J., Heslop, J. P. & Berridge, M. J. (1986) Nature (London) **320**, 631–634
Johanson, R. A., Hansen, C. A. & Williamson, J. R. (1988) J. Biol. Chem. **263**, 7465–7471
Kimura, Y., Hirata, M., Yamaguchi, K. & Koga, T. (1987) Arch. Biochem. Biophys. **257**, 363–369
Levin, R. M. & Weiss, B. (1976) Mol. Pharmacol. **12**, 581–589
Levin, R. M. & Weiss, B. (1978) Biochim. Biophys. Acta **540**, 197–204
Malnoë, A., Cox, J. A. & Stein, E. A. (1982) Biochim. Biophys. Acta **714**, 84–92
Mamar-Bachi, A. & Cox, J. A. (1988) Cell Calcium **8**, 473–482
Maulet, Y. & Cox, J. A. (1983) Biochemistry **22**, 5680–5686
Morris, A. J., Downes, C. P., Harden, T. K. & Michell, R. H. (1987) Biochem. J. **248**, 489–493
Prentki, M., Janjic, D. & Wollheim, C. B. (1983) J. Biol. Chem. **258**, 7597–7602
Ryu, S. H., Lee, S. Y., Lee, K.-Y. & Rhee, S. G. (1987) FASEB J. **1**, 388–393
Storey, D. J., Shears, S. B., Kirk, C. J. & Michell, R. H. (1984) Nature (London) **312**, 374–376
Takazawa, K., Passareiro, H., Dumont, J. E. & Erneux, C. (1988) Biochem. Biophys. Res. Commun. **153**, 632–641
Weber, G. (1975) Adv. Protein Chem. **29**, 1–93
Wyman, J., Jr. (1964) Adv. Protein Chem. **19**, 223–286
Yamaguchi, K., Hirata, M. & Kuriyama, H. (1987) Biochem. J. **244**, 787–791

Received 12 December 1988/8 February 1989; accepted 16 February 1989