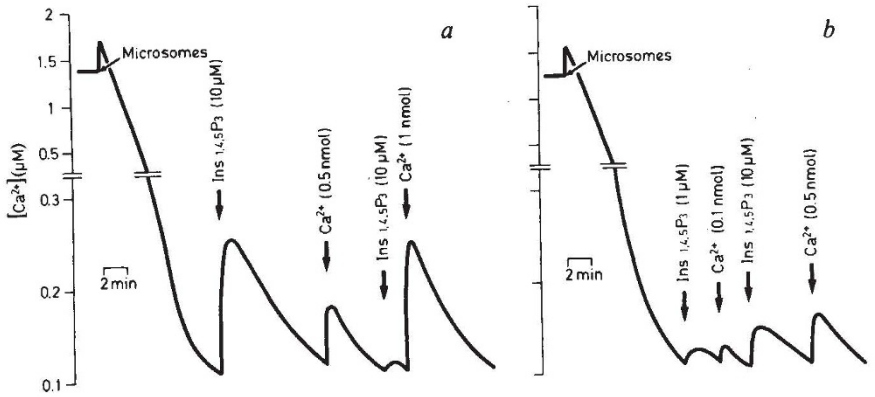


**Fig. 2** Extramicrosomal ambient free $Ca^{2+}$ concentration maintained by insulinoma microsomes: effect of Ins1,4,5$P_3$. Microsomes were incubated at 30 °C, *p*H 7.0 in 200 μl of a buffer containing 110 mM KCl, 2 mM $KH_2PO_4$, 25 mM HEPES, 1 mM $MgCl_2$, 1 mM MgATP, 3 mM creatine phosphate, 50 μg $ml^{-1}$ creatine kinase, 0.2 μM antimycin and 0.5 mg $ml^{-1}$ of bovine serum albumin, with continuous stirring. The medium [$Ca^{2+}$] was continuously recorded with a $Ca^{2+}$-selective minielectrode made and calibrated as described previously[22]. Where indicated, microsomes (0.75 mg protein $ml^{-1}$), Ins1,4,5$P_3$ or $CaCl_2$ (nmol per 200 μl) (in the amounts indicated) were added to the medium. Within 10 min, microsomes lowered the extramicrosomal ambient free $Ca^{2+}$ concentration to ~0.1 μM; they were not easily saturated with $Ca^{2+}$ as they could rapidly take up several sequential pulse additions (5 nmol per mg protein) of $Ca^{2+}$. The $Ca^{2+}$ accumulated could be rapidly released by adding the $Ca^{2+}$ ionophore A23187 (1 μg $ml^{-1}$) or by lowering the ATP present in the medium by the addition of glucose (5 mM) plus hexokinase (20 U $ml^{-1}$), indicating that $Ca^{2+}$ is accumulated into vesicular elements in a MgATP-dependent manner (not shown). If microsomes were preincubated with the $Ca^{2+}$ ionophore A23187 to empty the vesicles of $Ca^{2+}$, the addition of Ins1,4,5$P_3$ produced only a very small increase in medium [$Ca^{2+}$]. This amount of $Ca^{2+}$ corresponded to a similar increase in [$Ca^{2+}$] when Ins1,4,5$P_3$ was added to the incubation buffer alone; it averaged ~5–10% of the effect shown in *a* and was accounted for in the subsequent calculation. Ins1,4,5$P_3$ was obtained by alkaline hydrolysis of ox brain phosphatidylinositol-4,5-bisphosphate, followed by preparative paper chromatography (R.F.I. and M.J.B., in preparation). The figure shows representative experiments which were repeated at least eight times.

Three lines of evidence suggest that Ins1,4,5$P_3$ induces $Ca^{2+}$ release from the endoplasmic reticulum and not from plasma membrane vesicles contaminating the microsomal fraction. First, the insulinoma microsomal $Ca^{2+}$ uptake correlated well with the endoplasmic reticulum enzyme marker, but not at all with the plasma membrane marker. Second, the amount of $Ca^{2+}$ released by Ins1,4,5$P_3$ (10 μM) was 6.1 ± 0.2 nmol per mg protein (mean ± s.e. of six separate experiments). This is a considerable amount of $Ca^{2+}$—for comparison, most tissues, including pancreatic islets[1,2], have a total calcium content of ~20 nmol per mg protein. The $Ca^{2+}$ is, therefore, unlikely to originate quantitatively from contaminating plasma membrane vesicles, in a tissue that is particularly rich in reticular structures such as insulinoma cells. Third, Ins1,4,5$P_3$ mobilizes $Ca^{2+}$ in permeabilized pancreatic acinar and liver cells in which the plasma membrane should not function as a $Ca^{2+}$-storing organelle[19–21]. However, the possibility that Ins1,4,5$P_3$ could induce in intact cells an influx of external $Ca^{2+}$ should not be precluded.

Agonist-induced rises in intracellular free [$Ca^{2+}$] have been universally associated with an enhancement of both phosphatidylinositol turnover and polyphosphoinositide hydrolysis[3–18]. More recent observations of a resultant, rapid, $Ca^{2+}$-independent generation of water-soluble inositol phosphates, have led to the hypothesis that these compounds could act as intracellular messengers mobilizing cellular $Ca^{2+}$ stores[17,18]. This hypothesis is substantiated and extended by the present report. We have shown that Ins1,4,5$P_3$, at concentrations which are thought to occur in cells, can increase, within 20 s, the extramicrosomal concentration of $Ca^{2+}$ from 0.1 μM to ~0.2–0.3 μM, corresponding respectively to basal or stimulated conditions in a variety of tissues[24–26]. The results of the present study strongly suggest a specific role for Ins1,4,5$P_3$ as a cellular messenger inducing $Ca^{2+}$ release from the endoplasmic reticulum.

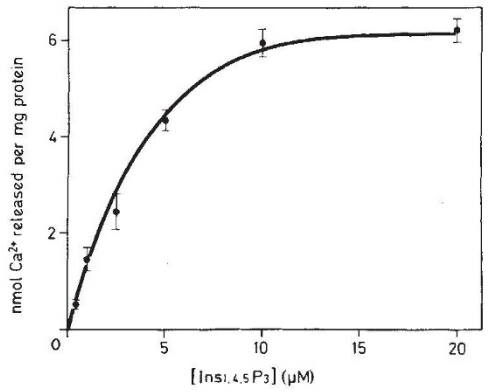


**Fig. 3** Dose–response of Ins1,4,5$P_3$-induced $Ca^{2+}$ release. Pulse additions of various known amounts of $CaCl_2$ (as shown in Fig. 2) were used to calibrate the $Ca^{2+}$ rapidly released by Ins1,4,5$P_3$. Values are the mean ± s.e. of three separate experiments.

We thank Nicole Challet, Janine Bassi and Charles Jorand for technical assistance. This work was supported by grant 3.246-082SR from the Swiss NSF.

Received 16 February; accepted 11 April 1984.

1. Borle, A. B. *Rev. Physiol. Biochem. Pharmac.* **90**, 14–153 (1981).
2. Wollheim, C. B. & Sharp, G. W. G. *Physiol. Rev.* **61**, 914–973 (1981).
3. Hokin, M. R. & Hokin, L. E. *J. biol. Chem.* **203**, 967–977 (1953).
4. Michell, R. H. *Biochim. biophys. Acta* **415**, 81–147 (1975).
5. Michell, R. H., Kirk, C. J., Jones, L. M., Downes, C. P. & Creba, J. A. *Phil. Trans. R. Soc.* **B296**, 123–133 (1981).
6. Berridge, M. J. *Molec. cell. Endocr.* **24**, 115–140 (1981).
7. Farese, R. V. *Metabolism* **32**, 628–641 (1983).
8. Best, L. & Malaisse, W. J. *Diabetologia* **25**, 299–305 (1983).
9. Abdel-Latif, A. A., Akhtar, R. A. & Hawthorne, J. N. *Biochem. J.* **162**, 61–73 (1977).
10. Kirk, C. J., Creba, J. A., Downes, P. & Michell, R. H. *Biochem. Soc. Trans.* **9**, 377–379 (1981).
11. Weiss, S. J., McKinney, J. S. & Putney, J. W. *Biochem. J.* **206**, 555–560 (1982).
12. Laychock, S. G. *Biochem. J.* **216**, 101–106 (1983).
13. Putney, J. W., Burgess, G. M., Halenda, S. P., McKinney, J. S. & Rubin, R. P. *Biochem. J.* **212**, 483–488 (1983).
14. Thomas, A. P., Marks, J. S., Coll, K. E. & Williamson, J. R. *J. biol. Chem.* **258**, 5716–5725 (1983).
15. Agranoff, B. W., Murthy, P. & Seguin, E. B. *J. biol. Chem.* **258**, 2076–2078 (1983).
16. Berridge, M. J., Dawson, R. M. C., Downes, C. P., Heslop, J. P. & Irvine, R. F. *Biochem. J.* **212**, 473–482 (1983).
17. Berridge, M. J. *Biochem. J.* **212**, 849–858 (1983).
18. Rebecchi, M. J. & Gershengorn, M. C. *Biochem. J.* **216**, 287–294 (1983).
19. Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. *Nature* **306**, 67–69 (1983).
20. Burgess, G. M. *et al. Nature* **309**, 63–66 (1984).
21. Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. *J. biol. Chem.* **259**, 3077–3081 (1984).
22. Prentki, M., Janjic, D. & Wollheim, C. B. *J. biol. Chem.* **258**, 7597–7602 (1983).
23. Downes, C. P., Mussat, M. C. & Michell, R. H. *Biochem. J.* **203**, 169–177 (1982).
24. Tsien, R. Y., Pozzan, T. & Rink, T. J. *Nature* **295**, 68–71 (1982).
25. Pozzan, T., Lew, D. P., Wollheim, C. B. & Tsien, R. Y. *Science* **221**, 1413–1415 (1983).
26. Wollheim, C. B. & Pozzan, T. *J. biol. Chem.* **259**, 2262–2267 (1984).
27. Chick, W. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 628–632 (1977).
28. Masiello, P. *et al. Endocrinology* **111**, 2091–2096 (1982).
29. Cooperstein, S. J. & Lazarow, A. *J. biol. Chem.* **189**, 665–670 (1951).
30. Sottocasa, G. L., Kuylenstierna, B., Ernster, L. & Bergstand, A. *J. Cell Biol.* **32**, 415–438 (1967).
31. Avruch, J. & Wallach, D. F. H. *Biochim. biophys. Acta* **233**, 334–347 (1971).
32. Herbert, V., Lau, K. S., Gottlieb, C. N. & Bleicher, S. J. *J. clin. Endocr. Metab.* **25**, 1375–1384 (1965).

# Corrigendum

## Therapeutic potential of monovalent monoclonal antibodies

**S. P. Cobbold & H. Waldmann**

*Nature* **308**, 460–462 (1984)

THE sentence on line 10 of the bold first paragraph should read: 'Starting from the original observation (by Glennie and Stevenson[3]) that rabbit antisera can . . . antigens'.