Glucose-induced accumulation of inositol trisphosphates in isolated pancreatic islets

Predominance of the 1,3,4-isomer

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Anion-exchange h.p.l.c. analysis of [^3H]inositol phosphates derived from glucose-stimulated isolated pancreatic islets that had been prelabelled with *myo*-[^3H]inositol revealed that the predominant inositol trisphosphate was the 1,3,4-isomer [$\text{Ins}(1,3,4)\text{P}_3$]. The 1,4,5-isomer [$\text{Ins}(1,4,5)\text{P}_3$] was also detectable, as was a more polar inositol phosphate with the chromatographic properties of inositol 1,3,4,5-tetrakisphosphate [$\text{Ins}(1,3,4,5)\text{P}_4$]. Glucose-induced accumulation of $\text{Ins}(1,3,4)\text{P}_3$ was augmented by Li^+ and occurred after maximal accumulation of $\text{Ins}(1,4,5)\text{P}_3$. These findings suggest a possible role for $\text{Ins}(1,3,4)\text{P}_3$ or its probable precursor $\text{Ins}(1,3,4,5)\text{P}_4$ in stimulus–secretion coupling in pancreatic islets.

INTRODUCTION

Rapidly accumulating evidence indicates that phospholipase C-catalysed hydrolysis of phosphatidylinositol 4,5-bisphosphate and the consequent generation of *myo*-inositol 1,4,5-trisphosphate [$\text{Ins}(1,4,5)\text{P}_3$] and diacylglycerol are important biochemical events in stimulus–response coupling in a variety of cells (Berridge & Irvine, 1984; Michell, 1986). Released $\text{Ins}(1,4,5)\text{P}_3$ is thought to induce a rise in the intracellular Ca^{2+} concentration by triggering mobilization of Ca^{2+} from the endoplasmic reticulum (Berridge, 1983) via interaction with a specific $\text{Ins}(1,4,5)\text{P}_3$ receptor (Spät *et al.*, 1986). Several examples are now recognized in which binding of an agonist to a cell-surface receptor results in activation of phospholipase C, and a GTP-binding factor appears to participate in coupling these events (Berridge, 1983; Litosch *et al.*, 1985; Martin *et al.*, 1986). Alternatively, activation of phospholipase C and $\text{Ins}(1,4,5)\text{P}_3$ release may be induced in some cells, such as cardiac myocytes, by membrane depolarization (Vergara *et al.*, 1985).

Glucose-induced insulin secretion from pancreatic-islet β -cells is a stimulus–response event in which $\text{Ins}(1,4,5)\text{P}_3$ accumulation may participate. Exogenous $\text{Ins}(1,4,5)\text{P}_3$ at concentrations between 1 and 10 μM induces release of $^{45}\text{Ca}^{2+}$ from the endoplasmic reticulum of digitonin-permeabilized islets (Wolf *et al.*, 1985) and of insulinoma cell lines (Biden *et al.*, 1984; Joseph *et al.*, 1984; Prentki *et al.*, 1984), and insulin secretion is stimulated in permeabilized islets by increasing the ambient free Ca^{2+} concentration within the submicromolar range (Colca *et al.*, 1985). At concentrations that stimulate insulin secretion, glucose appears to increase the cytosolic Ca^{2+} concentration in intact islet cells (Rorsman *et al.*, 1984; Wollheim & Sharp, 1981) and to induce the release from [^3H]inositol-labelled islets of material with the properties of an inositol trisphosphate on chromatography over Dowex AG1-X8 ion-exchange resin (Best & Malaisse, 1984; Montague *et al.*, 1985; Morgan *et al.*, 1985).

These chromatographic methods do not distinguish $\text{Ins}(1,4,5)\text{P}_3$ from inositol 1,3,4-trisphosphate [$\text{Ins}(1,3,4)\text{P}_3$]. By ion-exchange h.p.l.c. analysis, the latter isomer has been identified as the predominant inositol trisphosphate present after carbachol stimulation of rat parotid glands (Irvine *et al.*, 1984, 1985) and angiotensin stimulation of hepatocytes (Burgess *et al.*, 1985). Circumstantial evidence suggests that $\text{Ins}(1,3,4)\text{P}_3$, unlike $\text{Ins}(1,4,5)\text{P}_3$, does not induce release of Ca^{2+} from endoplasmic reticulum (Berridge & Irvine, 1984; Burgess *et al.*, 1985), but the possibility that $\text{Ins}(1,3,4)\text{P}_3$ is a second messenger in its own right with a different intracellular target has been suggested (Irvine *et al.*, 1984, 1985). $\text{Ins}(1,3,4)\text{P}_3$ appears to derive from hydrolysis of inositol 1,3,4,5-tetrakisphosphate [$\text{Ins}(1,3,4,5)\text{P}_4$], a compound which has been identified from carbachol-stimulated cerebral-cortical slices (Batty *et al.*, 1985) and which may also serve some so-far unidentified second-messenger function (Michell, 1986).

We have examined the isomeric composition of inositol phosphates from glucose-stimulated pancreatic islets labelled with [^3H]inositol and have found that, under conditions previously reported to induce [^3H] InsP_3 accumulation in islets, the predominant inositol trisphosphate is distinct from $\text{Ins}(1,4,5)\text{P}_3$ and has the chromatographic properties of $\text{Ins}(1,3,4)\text{P}_3$. A material with the chromatographic properties of InsP_4 has also been detected in glucose-stimulated pancreatic islets.

EXPERIMENTAL

Islets were isolated from the pancreata of adult (220 g) male Sprague–Dawley rats and cultured overnight as previously described (Turk *et al.*, 1984a,b). After washing three times in KRB buffer [115 mM-NaCl, 5.0 mM-KCl, 24 mM- NaHCO_3 , 1 mM- MgCl_2 , 2.5 mM- CaCl_2 , saturated with air/ CO_2 (19:1), pH 7.40] containing 3 mM-glucose, the cultured islets were incubated with 100 μCi of

Abbreviations used: *Ins*, *myo*-inositol; *Ins*1*P*, *myo*-inositol 1-phosphate; $\text{Ins}(1,4)\text{P}_2$, *myo*-inositol 1,4-bisphosphate; $\text{Ins}(1,3,4)\text{P}_3$, *myo*-inositol 1,3,4-trisphosphate; $\text{Ins}(1,4,5)\text{P}_3$, *myo*-inositol 1,4,5-trisphosphate; $\text{Ins}(1,3,4,5)\text{P}_4$, *myo*-inositol 1,3,4,5-tetrakisphosphate.

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myo-[1,2- ^3H]inositol (New England Nuclear; 59.6 Ci/mmol) for 90 min at 37 °C with shaking in 350 μl of KRB containing 25 mM-Hepes, 28 mM-glucose, and 0.1% (w/v) bovine serum albumin (Sigma). (Islets prelabelled at 3 mM-glucose exhibited similar ^3H incorporation and subsequent response to 28 mM-glucose.) The supernatant was then discarded and the islets were rinsed three times with KRB containing 3 mM-glucose. Islet incorporation of radiolabel averaged 20%. For each experimental condition, 500 islets were then placed in separate silane-treated vials with 1 ml of KRB containing 3 mM-glucose, 0 mM- or 10 mM-LiCl, and 0.1% albumin. After shaking at 37 °C for 30 min, the medium was removed; 1 ml of KRB containing 3 mM- or 28 mM-glucose was added; and shaking at 37 °C was continued for 15 s–5 min. Incubations were terminated by addition of ice-cold 15% (w/v) trichloroacetic acid (1 ml) and 50 μl of an internal-standard mixture containing 4 μg of AMP, 15 μg of ADP and 20 μg of ATP. Samples were vortex-mixed (15 s) and bath-sonicated (1 min) three times, followed by bath sonication (15 min) twice, at which point islet dissolution was complete, as assessed microscopically. Samples were then transferred to silane-treated (10 mm $\times$ 75 mm) centrifuge tubes, chilled on ice (20 min), and centrifuged (2000 g, 20 min). The supernatant was placed in a clean vial, extracted twice with diethyl ether (1 ml), adjusted to pH 7 with Tris (approx. 100 μl of 150 mg/ml), freeze-dried overnight, and reconstituted in 500 μl of 1 mM-EDTA, pH 7.0.

Samples were analysed by h.p.l.c. on a Partisil SAX-10 anion-exchange column (0.46 cm $\times$ 25 cm; Alltech, Deerfield, IL, U.S.A.) at a flow rate of 1 ml/min with continuous u.v. monitoring. The solvent program was: solvent A (water) for 5 min; a linear increase over 30 min to 60% solvent B (1.7 M-ammonium formate, adjusted to pH 3.7 with H_3PO_4); 60% solvent B (6 min); a linear gradient over 10 min to 100% solvent B (10 min); and 100% solvent B (20 min). Volumes of the collected fractions were 1 ml (0–40 min), 0.5 ml (40–50 min) and 1 ml (50–70 min). Samples (125 μl) of each fraction were subjected to liquid scintillation counting (5 min) in ACS (Amersham) scintillation fluid (3.5 ml) containing water (330 μl) and 1 M-HCl (88 μl). {Addition of water and HCl prevented the time-dependent diminution in radioactivity (c.p.m.) for standard *myo*-[2- ^3H]inositol 1,4,5-trisphosphate (Amersham; 5 Ci/mmol) that was otherwise observed when this compound was added to the scintillation cocktail as a solution in 1.0 M-ammonium formate, adjusted to pH 3.7 with H_3PO_4 .} Typically under these h.p.l.c. conditions, standard *myo*-[2- ^3H]inositol 1-phosphate (Amersham) was eluted in fraction 23; standard [^3H]Ins(1,3,4) P_3 was eluted in fractions 47–50; and standard AMP, ADP and ATP were eluted at fractions 22, 28 and 47 respectively. Some day-to-day variation in the exact elution volumes of these standard compounds was observed. After each run, the solvent composition was changed from 100% B to 0% B as a linear gradient over 10 min, and water was pumped through the column for 20 min before the next injection.

RESULTS

The conditions of anion-exchange h.p.l.c. analysis employed in this study have previously been demonstrated by Irvine *et al.* (1985) to distinguish *myo*-inositol

Fig. 1. Anion-exchange h.p.l.c. analysis of metabolites of [^3H]inositol from isolated pancreatic islets

Islets were isolated, cultured overnight, prelabelled with [^3H]inositol, incubated for 2 min with a stimulatory concentration (28 mM) of glucose and 10 mM-LiCl; radiolabelled products were recovered and analysed by h.p.l.c. as described in the Experimental section. As discussed in the text, IP_1 is considered to represent Ins1P, IP_2 Ins(1,4) P_2 , IP_{3a} Ins(1,3,4) P_3 , IP_{3b} Ins(1,4,5) P_3 , and IP_4 Ins(1,3,4,5) P_4 .

1-phosphate (Ins1P), *myo*-inositol 1,4-bisphosphate [Ins(1,4) P_2], Ins(1,3,4) P_3 and Ins(1,4,5) P_3 . In this system Ins1P, Ins(1,4) P_2 and Ins(1,3,4) P_3 are virtually co-eluted with AMP, ADP and ATP respectively, whereas Ins(1,4,5) P_3 is eluted just after Ins(1,3,4) P_3 . The ammonium formate concentration required to elute Ins(1,3,4,5) P_4 is about 50% higher than that required to elute Ins(1,3,4) P_3 (Batty *et al.*, 1985).

Fig. 1 illustrates the profile observed with this system upon analysis of the products obtained from isolated pancreatic islets that had been prelabelled with [^3H]inositol and then stimulated with 28 mM-glucose for 2 min in the presence of 10 mM-LiCl. The radiolabelled compound co-eluted with AMP is likely to be Ins1P and is designated IP_1 . Five peaks with polarity greater than that of Ins1P were observed reproducibly. The first (IP_2) virtually co-eluted with ADP and is probably Ins(1,4) P_2 . A less abundant material eluted just after Ins(1,4) P_2 may represent a positional isomer, such as Ins(1,3) P_2 . The predominant peak in the region of the chromatogram containing the inositol trisphosphates was virtually co-eluted with ATP, is designated IP_{3a} and corresponds to Ins(1,3,4) P_3 (see below). A much less abundant material, designated IP_{3b} , was eluted just after IP_{3a} and corresponds to Ins(1,4,5) P_3 (see below). Increasing the

Fig. 2. Distinction by h.p.l.c. between predominant inositol trisphosphate isomer from isolated pancreatic islets and $\text{Ins}(1,4,5)P_3$

(a) H.p.l.c. analysis of ATP and standard D-myo-[2- ^3H]inositol 1,4,5-trisphosphate obtained from Amersham. (b) H.p.l.c. analysis of the islet-derived material designated IP_{3a} in Fig. 1, which was recovered from the h.p.l.c. eluate, freeze-dried, and co-injected with ATP. (c) H.p.l.c. analysis of co-injected ATP, islet-derived IP_{3a} and standard [^3H]Ins(1,4,5) P_3 . IP_{3a} is considered to correspond to $\text{Ins}(1,3,4)P_3$ (see the text).

Table 1. Radioactivity from [^3H]inositol migrating on h.p.l.c. as inositol metabolites from prelabelled pancreatic islets incubated with 3 mM- or 28 mM-glucose for 2 min

Islets (500 for each condition) were isolated, prelabelled with [^3H]inositol, washed free of unincorporated radiolabel, preincubated for 30 min at 37 °C with 3 mM-glucose, and then incubated for 2 min at 37 °C with 3 mM- or 28 mM-glucose and 10 mM-LiCl as described in the Experimental section. Products were recovered and analysed by h.p.l.c. as described in Fig. 1. The total radioactivity (c.p.m.) in each peak was corrected for background. The net d.p.m. in the peak for each compound derived from incubations at 28 mM-glucose was divided by that at 3 mM-glucose in the same experiment to yield the 'Ratio 28 mM/3 mM' tabulated. All tabulated values represent means $\pm$ S.E.M. for six experiments. The structures assigned to IP_{3a} and IP_{3b} are given in the legend to Fig. 1.

Compound	^3H (d.p.m.)		Ratio 28 mM/3 mM
	3 mM-glucose	28 mM-glucose	
Inositol	$(99.64 \pm 19.12) \times 10^3$	$(99.87 \pm 4.83) \times 10^3$	1.03 ± 0.18
IP_1	$(37.14 \pm 9.14) \times 10^3$	$(36.79 \pm 6.22) \times 10^3$	1.13 ± 0.14
IP_2	$(13.27 \pm 2.91) \times 10^2$	$(21.25 \pm 4.28) \times 10^2$	1.74 ± 0.24
IP_{3a}	$(13.34 \pm 4.67) \times 10^2$	$(22.69 \pm 4.00) \times 10^2$	2.24 ± 0.54
IP_{3b}	$(2.46 \pm 0.73) \times 10^2$	$(3.71 \pm 0.99) \times 10^2$	1.87 ± 0.48
IP_4	$(3.20 \pm 2.24) \times 10^2$	$(3.39 \pm 1.24) \times 10^2$	1.16 ± 0.23

ammonium formate concentration resulted in elution of a more polar compound, which probably represents $\text{Ins}(1,3,4,5)P_4$.

To establish that the predominant inositol trisphosphate from these glucose-stimulated pancreatic islets was distinct from $\text{Ins}(1,4,5)P_3$, the chromatographic behaviour of islet-derived IP_{3a} was compared with that of standard [^3H]Ins(1,4,5) P_3 obtained from Amersham, as illustrated in Fig. 2. The standard [^3H]Ins(1,4,5) P_3 was eluted just after co-injected ATP, as reported by Irvine *et al.* (1985). Islet-derived IP_{3a} that had been harvested from the initial h.p.l.c. analysis, concentrated by

freeze-drying and re-injected along with ATP was virtually co-eluted with ATP. Co-injection of h.p.l.c.-purified islet-derived IP_{3a} with standard [^3H]Ins(1,4,5) P_3 produced two peaks, indicating that the two materials were not identical. Similar experiments with islet-derived IP_{3b} demonstrated that this material was co-eluted with standard [^3H]Ins(1,4,5) P_3 .

The effects on islet inositol phosphate production of 2 min of exposure to 3 mM- or 28 mM-glucose in the presence of 10 mM-LiCl were next compared, as summarized in Table 1. The higher glucose concentration stimulates insulin secretion from islets (Turk *et al.*,

Fig. 3. Time course of glucose-induced inositol phosphate accumulation in isolated pancreatic islets

Incubations were performed as described in Table 1, except that the incubation period was varied between 30 s and 5 min. Products were recovered and analysed as described in Fig. 1. The parameter plotted is the 'Ratio 28 mM/3 mM' described in Table 1. An asterisk indicates that the observed ratio is greater than 1.0 by more than 2 S.E.M. IP_{3a} and IP_{3b} correspond to Ins(1,3,4)P₃ and Ins(1,4,5)P₃ respectively (see the text).

1984a,b) and also caused accumulation of larger amounts of [³H]IP₂, [³H]IP_{3a} and [³H]IP_{3b} than were observed at the lower glucose concentration. When the radioactivity (d.p.m.) in each inositol phosphate peak at 28 mM-glucose was divided by that at 3 mM-glucose for each experiment, this ratio ('Ratio 28 mM/3 mM') differed from 1.0 by more than 2 S.E.M. for IP_{3a} and IP₂. In the absence of LiCl, glucose-induced accumulation of IP_{3a} was also observed (Ratio 28 mM/3 mM = 3.42), but the absolute amount of radioactivity in the IP_{3a} peak was diminished by about 5-fold and that in the IP_{3b} peak was unaffected. This observation supports the assessment of the identity of IP_{3a} as Ins(1,3,4)P₃ and of IP_{3b} as Ins(1,4,5)P₃, since Burgess *et al.* (1985) have demonstrated that Li⁺ augments accumulation of Ins(1,3,4)P₃, but not of Ins(1,4,5)P₃, in pancreatic acinar cells.

The time course of glucose-induced inositol phosphate accumulation in the islets is illustrated in Fig. 3. A glucose-induced rise in IP_{3b} accumulation was observed as early as 30 s after exposure to 28 mM-glucose, and a further rise was observed at 1 min, at which time the 'Ratio 28 mM/3 mM' for IP_{3b} was greater than 1.0 by more than 2 S.E.M. No further increase in IP_{3b} accumulation was observed at later time points. Glucose-induced accumulation of IP_{3a} was also observed, but occurred later and continued to increase at 2 min and at 5 min after exposure to 28 mM-glucose. This observation also supports the assignment of the identity of IP_{3a} of Ins(1,3,4)P₃ and that of IP_{3b} as Ins(1,4,5)P₃, since Burgess *et al.* (1985) have demonstrated that accumulation of Ins(1,4,5)P₃ occurs early after angiotensin stimulation of hepatocytes, then decreases slightly, and finally achieves a stable, modestly elevated, plateau.

Ins(1,3,4)P₃ accumulation occurs slightly later and continues to increase progressively with time in that system (Burgess *et al.*, 1985).

As also illustrated in Fig. 3, shortly after the rise in IP_{3a}, glucose-induced accumulation of IP₂ was also observed. The time course of IP₄ accumulation in the glucose-stimulated islets roughly followed the temporal pattern of Ins(1,3,4,5)P₄ accumulation demonstrated by Batty *et al.* (1985) in carbachol-stimulated brain slices, with an early rise, followed by a decline, and then a secondary rise.

DISCUSSION

The observations reported here confirm previous demonstrations (Best & Malaisse, 1984; Montague *et al.*, 1985; Morgan *et al.*, 1985) that concentrations of glucose that stimulate insulin secretion also induce polyphosphoinositide hydrolysis and inositol trisphosphate accumulation in isolated pancreatic islets. The new information contributed by the present study is that the predominant inositol trisphosphate accumulating in islets under the reported conditions of stimulation is Ins(1,3,4)P₃ and not Ins(1,4,5)P₃. The identification of this material as Ins(1,3,4)P₃ is supported by its chromatographic behaviour relative to standard Ins(1,4,5)P₃ and ATP, by the temporal pattern of its accumulation relative to Ins(1,4,5)P₃, by the Li⁺-induced augmentation of its accumulation, and by the detection of its probable precursor Ins(1,3,4,5)P₄ in islets.

The physiological significance of Ins(1,3,4)P₃ is at present unknown, although its accumulation during the response to various stimuli has been reported in parotid

gland (Irvine *et al.*, 1984, 1985), hepatocytes, pancreatic acinar cells, human myelomonocytic leukaemia cells (Burgess *et al.*, 1985), cerebral-cortical slices (Batty *et al.*, 1985), and now in isolated pancreatic islets. The observations that Li^+ augments accumulation of $\text{Ins}(1,3,4)\text{P}_3$, but not of $\text{Ins}(1,4,5)\text{P}_3$, and that Li^+ in general fails to augment Ca^{2+} -mediated responses to signals, have been taken to imply that $\text{Ins}(1,3,4)\text{P}_3$ is not involved in intracellular Ca^{2+} mobilization (Burgess *et al.*, 1985). It may be that $\text{Ins}(1,3,4)\text{P}_3$ represents an inactivation product of its probable precursor $\text{Ins}(1,3,4,5)\text{P}_4$, which is converted into $\text{Ins}(1,3,4)\text{P}_3$ by a Li^+ -insensitive 5-phosphomonoesterase. Although it is possible that $\text{Ins}(1,3,4,5)\text{P}_4$ will prove to have a second-messenger function, its biological activities are also at present unknown (Michell, 1986).

Morgan *et al.* (1985) have reported a poor correspondence between glucose-induced inositol trisphosphate accumulation and $^{45}\text{Ca}^{2+}$ fluxes in isolated islets and have suggested the possibility that this may reflect predominant accumulation of $\text{Ins}(1,3,4)\text{P}_3$ rather than $\text{Ins}(1,4,5)\text{P}_3$. Our observations support this hypothesis. Our data also indicate, however, that glucose does stimulate $\text{Ins}(1,4,5)\text{P}_3$ accumulation. Moreover, the progressive glucose-induced accumulation of $\text{Ins}(1,3,4)\text{P}_3$ in the presence of Li^+ seems to imply that the rate of $\text{Ins}(1,4,5)\text{P}_3$ generation remains higher in islets incubated with 28 mM-glucose than in islets with 3 mM-glucose for at least 5 min. This assumes that $\text{Ins}(1,3,4)\text{P}_3$ is generated via the sequential actions of an $\text{Ins}(1,4,5)\text{P}_3$ 3-kinase and of an $\text{Ins}(1,3,4,5)\text{P}_4$ 5-phosphomonoesterase, as seems likely (Batty *et al.*, 1985; Michell, 1986). $\text{Ins}(1,4,5)\text{P}_3$ may therefore participate in the increase in islet intracellular Ca^{2+} believed to occur on glucose stimulation (Rorsman *et al.*, 1984), possibly in concert with other Ca^{2+} -mobilizing substances, including arachidonic acid (Wolf *et al.*, 1986).

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REFERENCES

- Batty, I. R., Nahorski, S. R. & Irvine, R. F. (1985) *Biochem. J.* **232**, 211–215
- Berridge, M. J. (1983) *Biochem. J.* **212**, 849–858
- Berridge, M. J. & Irvine, R. F. (1984) *Nature (London)* **312**, 315–321
- Best, L. & Malaisse, W. J. (1984) *Endocrinology (Baltimore)* **115**, 1814–1820
- Biden, T. J., Prentki, M., Irvine, R. F., Berridge, M. J. & Wollheim, C. B. (1984) *Biochem. J.* **223**, 467–473
- Burgess, G. M., McKinney, J. S., Irvine, R. F. & Putney, J. W. (1985) *Biochem. J.* **232**, 237–243
- Colca, J. R., Wolf, B. A., Comens, P. G. & McDaniel, M. A. (1985) *Biochem. J.* **228**, 529–536
- Irvine, R. F., Letcher, A. J., Lander, D. J. & Downes, C. P. (1984) *Biochem. J.* **223**, 237–243
- Irvine, R. F., Ånggård, E. E., Letcher, A. J. & Downes, C. P. (1985) *Biochem. J.* **229**, 505–511
- Joseph, S. K., Williams, R. J., Corkey, B. E., Matschinsky, F. M. & Williamson, J. R. (1984) *J. Biol. Chem.* **259**, 12952–12955
- Litosch, I., Wallis, C. & Fain, J. N. (1985) *J. Biol. Chem.* **260**, 5464–5471
- Martin, T. F. J., Lucas, D. O., Bajjalieh, S. M. & Kowalchyk, J. A. (1986) *J. Biol. Chem.* **261**, 2918–2927
- Michell, R. (1986) *Nature (London)* **319**, 176–177
- Montague, W., Morgan, N. G., Rumford, G. M. & Prince, C. A. (1985) *Biochem. J.* **227**, 483–489
- Morgan, N. G., Rumford, G. M. & Montague, W. (1985) *Biochem. J.* **228**, 713–718
- Prentki, M., Biden, T. J., Janjic, D., Irvine, R. F., Berridge, M. J. & Wollheim, C. B. (1984) *Nature (London)* **309**, 562–564
- Rorsman, P., Abrahamsson, H., Gylfe, E. & Hellman, B. (1984) *FEBS Lett.* **170**, 196–200
- Spät, A., Bradford, P. G., McKinney, J. S., Rubin, R. P. & Putney, J. W. (1986) *Nature (London)* **319**, 514–516
- Turk, J., Colca, J. R., Kotagal, N. & McDaniel, M. L. (1984a) *Biochim. Biophys. Acta* **794**, 110–124
- Turk, J., Colca, J. R., Kotagal, N. & McDaniel, M. L. (1984b) *Biochim. Biophys. Acta* **794**, 125–136
- Vergara, J., Tsien, R. Y. & Delay, M. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6351–6356
- Wolf, B. A., Comens, P. G., Ackermann, K. E., Sherman, W. R. & McDaniel, M. L. (1985) *Biochem. J.* **227**, 965–969
- Wolf, B. A., Turk, J., Sherman, W. R. & McDaniel, M. L. (1986) *J. Biol. Chem.* **261**, 3501–3511
- Wollheim, C. B. & Sharp, G. W. P. (1981) *Physiol. Rev.* **61**, 914–973

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