Inositol 1,4,5-trisphosphate mobilizes intracellular Ca^{2+} from permeabilized insulin-secreting cells

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A possible role in secretory processes is proposed for inositol 1,4,5-trisphosphate (IP_3), based upon investigations of the Ca^{2+} steady state maintained by 'leaky', insulin-secreting RINm5F cells. These cells had been treated with digitonin to permeabilize their plasma membranes and thereby ensure that only intracellular Ca^{2+} buffering mechanisms were active. When placed in a medium with a cation composition resembling that of the cytosol, cells rapidly took up Ca^{2+} as measured by a Ca^{2+} -specific minielectrode. Two Ca^{2+} steady states were observed. A lower level of around 120 nM required ATP-dependent Ca^{2+} uptake and was probably determined by the endoplasmic reticulum. The higher steady state (approx. 800 nM), seen only in the absence of ATP, was shown to be due to mitochondrial activity. IP_3 specifically released Ca^{2+} accumulated in the ATP-dependent pool, but not from mitochondria, since Ca^{2+} release was demonstrated in the presence of the respiratory poison antimycin. The IP_3 -induced Ca^{2+} release was rapid, with 50% of the response being seen within 15 s. The apparent K_m was 0.5 μM and maximal concentrations of IP_3 (2.5 μM) produced a peak Ca^{2+} release of 10 nmol/mg of cell protein, which was followed by re-uptake. A full Ca^{2+} response was seen if sequential pulses of 2.5 μM - IP_3 were added at 20 min intervals, although there was a slight (<20%) attenuation if the intervening period was decreased to 10 min. These observations could be related to the rate of IP_3 degradation which, in this system, corresponded to a 25% loss of added ^{32}P label within 2 min, and a 75% loss within 20 min. The results suggest that IP_3 might act as a link between metabolic, cationic and secretory events during the stimulation of insulin release.

The redistribution of intracellular Ca^{2+} seen in many cells in response to agonist-receptor binding is associated with hydrolysis of membrane phosphoinositides (Michell, 1975; Michell *et al.*, 1981). More specifically, a rapid rise has been recently reported in inositol 1,4,5-trisphosphate (IP_3), the water-soluble moiety derived from phosphatidylinositol 4,5-bisphosphate (Agranoff *et al.*, 1983; Berridge *et al.*, 1983; Berridge, 1983;

Martin, 1983; Rebecchi & Gershengorn, 1983; Downes & Wusteman, 1983; Burgess *et al.*, 1984; Schlegel *et al.*, 1984). Moreover, in permeabilized cell preparations of the exocrine pancreas (Streb *et al.*, 1983), and liver (Burgess *et al.*, 1984; Joseph *et al.*, 1984) IP_3 mobilizes Ca^{2+} from an intracellular pool, recently identified as the endoplasmic reticulum (Prentki *et al.*, 1984a). These observations, and earlier reports that receptor activation in these tissues is associated with a rise in cytosolic Ca^{2+} (Ochs *et al.*, 1983; Charest *et al.*, 1983), together suggest that IP_3 may act as an intracellular messenger mediating the physiological responses of agonist binding.

Nutrient-induced insulin release, which is de-

Abbreviations used: PIP_2 , phosphatidylinositol 4,5-bisphosphate; IP_1 , inositol 1-phosphate; IP_2 , inositol 1,4-bisphosphate; IP_3 , inositol 1,4,5-trisphosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

pendent upon metabolism of the initiator and is therefore receptor-independent (Malaisse, 1984), is also associated with an increase in cytosolic Ca^{2+} (Wollheim & Pozzan, 1984). This calcium signal, which is an integral component of the secretory process, is thought to be derived, at least in part, from the mobilization of intracellular Ca^{2+} stores (Wollheim & Sharp, 1981; Wollheim & Pozzan, 1984). Furthermore, the stimulation of insulin release by the nutrient secretagogue glucose is associated with increased phospholipid turnover (Best & Malaisse, 1983), and in particular with the hydrolysis of PIP_2 (Laychock, 1983). More specifically we have recently shown that D-glyceraldehyde, which acts as a nutrient stimulus in the insulin-secreting RINm5F cell line (Praz *et al.*, 1983; Wollheim & Pozzan, 1984), also elevates the IP_3 concentration in those cells within 1 min of addition (Biden *et al.*, 1984).

In the following experiments, we have used digitonin-permeabilized RINm5F cells and Ca^{2+} -specific minielectrodes to characterize further the mobilization of intracellular Ca^{2+} by IP_3 , and hence to implicate its involvement in processes independent of receptor activation.

Experimental

Materials

IP_3 and IP_2 were produced by the alkaline hydrolysis of ox brain PIP_2 and PIP and purified by preparative paper chromatography (Irvine *et al.*, 1984). Inositol 1,[4,5- ^{32}P]trisphosphate was isolated from ^{32}P -labelled erythrocyte ghosts as previously described (Downes *et al.*, 1982). Inositol 2-phosphate and *myo*-inositol were purchased from Sigma. All other analytical grade chemicals were supplied from the same source or from Fluka or Merck. The sources of tissue culture materials were as previously described (Praz *et al.*, 1983).

Culture and permeabilization of cells

The long-term maintenance of the RINm5F cells and their transferral to spinner culture have been previously described in detail (Praz *et al.*, 1983; Wollheim & Pozzan, 1984). Cells (60×10^6) were harvested by centrifugation (80g, 10 min) and washed twice with solution A containing KCl (125 mM), Hepes (25 mM), KH_2PO_4 (2 mM), MgATP (0.5 mM), EGTA (0.25 mM) and bovine serum albumin (1 mg/ml). The pH was adjusted to 7.0 with KOH and the solution kept on ice. The cells were resuspended in two 4 ml portions of solution A (approx. 5×10^6 cells/ml), and allowed to equilibrate for 5 min at 37°C before the addition of a 100-fold concentrated solution of digitonin (final concn. 10 μM). After exactly 4 min a 3-fold excess of ice-cold solution B was added. This was

identical in composition to solution A but without EGTA, and was used for all subsequent resuspensions. The cells were centrifuged (160g, 5 min), the pellets combined and washed once more. For a final wash the cells were transferred to disposable 3 ml centrifuge tubes and centrifuged at 80g for 5 min. The pellet was resuspended in 50 μl to give a concentration of $(1-3) \times 10^6$ cells/10 μl and kept on ice until use. After treatment with digitonin and the subsequent washes $77 \pm 2\%$ ($n = 10$) of the cells were unable to exclude Trypan Blue. Permeabilization was apparently irreversible because these cells maintained similar Ca^{2+} steady states even up to several hours after treatment. This contrasts with control cells washed in a Krebs-Ringer Hepes medium (Wollheim & Pozzan, 1984), more than 95% of which excluded Trypan Blue, and which were incapable of lowering ambient free Ca^{2+} . In further control experiments designed to mimic completely the permeabilization procedure except for the omission of digitonin during the incubation period, $29 \pm 4\%$ ($n = 3$) of the cells took up Trypan Blue, but they were able to lower ambient free Ca^{2+} only slowly and incompletely as compared with the digitonin-treated cells.

Measurement of ambient free Ca^{2+}

The preparation and calibration of the Ca^{2+} -specific minielectrodes used in this study, as well as details of the experimental procedure, have been previously described (Prentki *et al.*, 1983). Incubations were carried out at 30°C and pH 7.0 in 200 μl of a medium containing KCl (110 mM), NaCl (10 mM), KH_2PO_4 (2 mM), MgCl_2 (1 mM), Hepes (25 mM), succinate (5 mM), oligomycin (1 $\mu\text{g}/\text{ml}$) and bovine serum albumin (0.5 mg/ml). In many experiments MgATP (2 mM), and an ATP-regenerating system of phosphocreatine (5 mM) and creatine kinase (20 units/ml) were also present. Other additions are described in the Figure legends. Cells were added to a final concn. of $(1-5) \times 10^6$ cells/200 μl . The figures shown here are representative of experiments repeated at least three times.

Measurement of IP_3 degradation

Degradation experiments were carried out in the same incubation vessel and under exactly the same conditions as for measurement of ambient free Ca^{2+} . Cells were added to 200 μl of the standard incubation medium, containing in addition MgATP (2 mM) and the ATP-regenerating system. After 10 min equilibration inositol 1,[4,5- ^{32}P]trisphosphate was added (diluted with unlabelled IP_3 to a final concn. of 2.5 μM), and 50 μl samples were withdrawn at 0, 2, 10 and 20 min. The aliquots were added to 10% (w/v) trichloroacetic acid and washed five times with ether. The

aqueous layer was neutralized with NaOH (1M) and transferred to columns containing Dowex 1X8 (formate form), and eluted exactly as described previously (Berridge *et al.*, 1983). Radioactivity in the fractions was quantified by liquid-scintillation counting with 67% (v/v) Aquasol 2 (New England Nuclear).

Results

Characterization of the two Ca²⁺ steady states maintained by permeabilized RINm5F cells

The addition of permeabilized cells to the basic incubation medium containing an ATP-regenerating system lowered the medium free Ca²⁺ within 10–15 min to a new steady state of around 120 nM (119 ± 3 nM; *n* = 10). This level was independent of the initial Ca²⁺ concentration of the medium (range 1.1–8.6 μM) (results not shown), and was also restored after addition of exogenous Ca²⁺ (Fig. 1). Attainment of this steady state depended upon Ca²⁺ uptake into an ATP-dependent pool (Fig. 2a), and was no longer maintained when ATP in the medium was markedly lowered by the addition of glucose plus hexokinase (Fig. 1). Moreover, it was insensitive to the mitochondrial poison antimycin (Fig. 2a), and was therefore most probably determined by Ca²⁺-

Fig. 1. Effects of IP₃ on the steady state free Ca²⁺ concentration maintained by digitonin-permeabilized RINm5F cells

Cells were incubated at 30°C and pH 7.0 in 200 μl of a buffer containing 110 mM-KCl, 10 mM-NaCl, 2 mM-KH₂PO₄, 1 mM-MgCl₂, 25 mM-Hepes, 5 mM-succinate, 2 mM-MgATP, 5 mM-phosphocreatine, 20 units of creatine kinase/ml, 1 μg of oligomycin/ml and 0.5 mg of bovine serum albumin/ml. At the points indicated Ca²⁺ (1.5 nmol) or IP₃ (2.5 μM final concn.) were added. HK indicates the addition of hexokinase (2 units/ml) plus glucose (8.3 mM). Cell (3.7 × 10⁶) were added at the point marked by the thick arrow (final concn. 2 mg of protein/ml).

buffering systems associated with the endoplasmic reticulum.

The higher Ca²⁺ steady state of around 800 nM (780 ± 31; *n* = 4) was dependent upon the respiratory substrate succinate (results not shown), but was only seen in the absence of ATP (cf. Figs. 1 and 2). It was immediately abolished by antimycin (Fig. 2a), but was completely unaffected by the presence of the Ca²⁺-ATPase inhibitor vanadate (Fig. 2b). This was a bidirectional Ca²⁺ 'set point' since it was restored after either raising the free Ca²⁺ concentration (by addition of exogenous Ca²⁺), or lowering it (by addition of the Ca²⁺ chelator EGTA) (Figs. 2a and 2b). The maintenance of this level is controlled by the mitochondria.

IP₃ promoted Ca²⁺ release

When added to cells maintaining the lower Ca²⁺ steady state, IP₃ promoted a rapid Ca²⁺ release, 50% of the response being seen within 15 s. The released Ca²⁺ was then slowly taken back up by the cells. The response to subsequent IP₃ additions

Fig. 2. Comparison of IP₃-induced Ca²⁺ release at the two Ca²⁺ steady state levels

Cells were incubated under conditions identical to those described in Fig. 1, except for omission of ATP and the ATP-regenerating system, and the addition of ADP (0.2 mM), glucose (8.3 mM) and hexokinase (2 units/ml). At the points indicated Ca²⁺, 1 nmol (a) or 0.5 nmol (b), was added, as well as IP₃ (2.5 μM) and EGTA (1.0 nmol). Ant refers to the addition of antimycin (0.2 μM), and ATP to the addition of 5 mM-MgATP plus 10 mM-phosphocreatine and 40 units of creatine kinase/ml. (a) Cells (3.7 × 10⁶) were added at the point marked by the thick arrow (final concn. 2 mg of protein/ml). (b) Vanadate (120 μM) was present from the beginning of the incubation. Cells (3.8 × 10⁶) were added where indicated by the thick arrow (final concn. 2.2 mg of protein/ml).

was slightly attenuated (Fig. 1). This effect was time-dependent since a second peak was $18 \pm 3\%$ lower when IP_3 was added around 10 min after the first [$n = 5$; chamber content of $(3.0 \pm 0.4) \times 10^6$ cells]. However, the response to a second addition was restored to $102 \pm 1\%$ of the first when the intervening period was extended to 20 min [$n = 4$; $(3.1 \pm 0.3) \times 10^6$ cells]. The source of the released Ca^{2+} was truly vesicular, since IP_3 had no effect after depletion of the stores with the Ca^{2+} ionophore A23187 ($5 \mu\text{g/ml}$) (results not shown). It should also be noted that a calibrating Ca^{2+} addition at the higher steady state gives a much larger peak (Fig. 1). This can be simply explained by a greater saturation of Ca^{2+} -binding sites at higher resting Ca^{2+} concentrations, which would then enable most of the added pulse to remain in the ionized form. Hence added Ca^{2+} gives a larger peak in the presence of an ion like Co^{2+} (0.1 mM) which competes for the binding sites (results not shown).

The average release at the mitochondrial set point was $0.9 \pm 0.2 \text{ nmol of Ca}^{2+}/\text{mg of protein}$ ($n = 4$) in response to $2.5 \mu\text{M-IP}_3$, as opposed to $10.0 \pm 1.9 \text{ nmol of Ca}^{2+}/\text{mg of protein}$ ($n = 4$) at the lower steady state. This suggests that, prior to release, Ca^{2+} uptake into an ATP-dependent pool is needed. The small effect seen in Fig. 2(a) would then be explained by the partial filling of this pool

Fig. 3. Concentration-dependence of IP_3 -induced Ca^{2+} release

Cells were incubated under conditions identical to those described in Fig. 1. The responses to IP_3 were calibrated by additions of known portions of Ca^{2+} to give equivalent peak heights. Points represent means $\pm$ S.E.M. of three or four individual determinations from a total of nine different cell preparations [average cell addition $(2.9 \pm 0.4) \times 10^6$]. The maximal release corresponds to $11.0 \pm 1.4 \text{ nmol of Ca}^{2+} \text{ released/mg of cell protein}$.

due to the presence of a low contaminating concentration of ATP. When this residual uptake was blocked from the beginning of the experiment by the ATPase inhibitor vanadate, the IP_3 -induced Ca^{2+} release at the higher steady state was completely abolished (Fig. 2b).

In a separate experiment, IP_3 was able to release Ca^{2+} normally when vanadate ($120 \mu\text{M}$) was added after the ATP-dependent uptake was complete, and the cells had achieved the lower Ca^{2+} steady state (results not shown). Release was similarly unaffected by the immediate prior addition of either nifedipine ($5 \mu\text{M}$), verapamil ($10 \mu\text{M}$) or cobalt ($100 \mu\text{M}$), which block voltage-dependent Ca^{2+} channels (Tan & Tashjian, 1984), or by dantrolene ($30 \mu\text{M}$) or Ruthenium Red ($20 \mu\text{M}$), which at these concentrations inhibit Ca^{2+} release from sarcoplasmic reticulum (Desmedt & Hainaut, 1979; Meissner, 1984).

The concentration dependence of the IP_3 effect is shown in Fig. 3. The maximal response, which was attained with $2.5 \mu\text{M}$, corresponded to a Ca^{2+} release of approx. $10 \text{ nmol/mg of protein}$. The apparent K_m was slightly more than $0.5 \mu\text{M-IP}_3$. The ability to release Ca^{2+} appears specific for IP_3 , since there was no detectable effect with either IP_2 or *myo*-inositol (both $2.5 \mu\text{M}$) or with inositol 2-phosphate ($7.5 \mu\text{M}$) (results not shown).

Degradation of IP_3

The degradation of exogenous labelled IP_3 was studied under conditions identical with those used for the measurement of free Ca^{2+} concentrations, and employed similar numbers of permeabilized cells. As can be calculated from the data shown in Fig. 4, around 25% of the added IP_3 was dephosphorylated within 2 min, giving an initial degradation rate of about $0.2 \text{ nmol/min per mg of protein}$. By 10 min more than 60% had been degraded, rising to greater than 75% degradation at 20 min. It should be noted that the hydrolysis of IP_3 may well be faster than suggested by these data, because in similar experiments with permeabilized pancreatic acinar cells, some incorporation of ^{32}P into ATP has been observed (R. F. Irvine, H. Streb, I. Schulz & M. J. Berridge, unpublished work). The stepwise elution of anion-exchange columns, as specifically described for separation of IP_1 and IP_2 , did not allow quantitative resolution of IP_2 from P_i , so the sum of these two breakdown products is displayed in Fig. 4. It is apparent that the increase in these two, water-soluble, degradation products account almost completely for the decrease in IP_3 radioactivity. The elution of IP_3 itself from the ion-exchange columns gave a $88 \pm 4\%$ ($n = 3$) yield, as judged by the recovery of known samples of ^{32}P -labelled IP_3 .

Fig. 4. Degradation of ³²P-labelled IP₃

Cells were incubated under conditions identical to those described in Fig. 1. After 10 min equilibration, inositol 1-[4,5-³²P]trisphosphate, together with IP₃, was added to give a final concn. of 2.5 μM. For further details see the Experimental section. Each point represents the mean ± S.E.M. of four individual determinations [average cell number (2.6 ± 0.2) × 10⁶]. —, Radioactivity in IP₃; ----, radioactivity in IP₂ plus P_i.

Discussion

Cells treated with digitonin or saponin have been previously used to study Ca²⁺ homeostasis in the liver and exocrine pancreas (Becker *et al.*, 1980; Murphy *et al.*, 1980; Streb & Schulz, 1983; Burgess *et al.*, 1983). Even though the use of permeabilized cells effectively bypasses the Ca²⁺ regulatory mechanisms operating across the plasma membrane, the Ca²⁺ steady state of approx. 120 nM is remarkably similar to the resting free Ca²⁺ concentration of 105 nM, as recently determined in intact RINm5F cells using the fluorescent indicator quin2 (Wollheim & Pozzan, 1984). This excellent agreement suggests that intracellular structures play an important role in determining the resting Ca²⁺ level in intact cells. This is consistent with conclusions reached in other cell types (Becker *et al.*, 1980; Streb & Schulz, 1983).

In permeabilized cell preparations the main structures regulating the Ca²⁺ steady state are likely to be endoplasmic reticulum, mitochondria and/or secretory granules. The higher set point reported here, which is maintained in the absence of ATP, is mitochondrial since it was dependent upon respiratory substrate and was abolished by the mitochondrial poison antimycin. Moreover, this setpoint is quantitatively very similar to that maintained, under identical experimental conditions, by isolated mitochondria derived from a rat insulinoma (Prentki *et al.*, 1983).

There is strong evidence that the lower steady state is determined by the endoplasmic reticulum. Firstly, both the attainment and the maintenance of this level were dependent upon ATP, which is consistent with the known Mg²⁺-dependent Ca²⁺-ATPase responsible for the Ca²⁺ uptake by endoplasmic and sarcoplasmic reticulum (Borle, 1981). Secondly, the lower steady state was not mitochondrially regulated (see above). Thirdly, secretory granules seem an unlikely candidate because a granule fraction derived from a rat insulinoma, although containing a lot of vesicular Ca²⁺, was unable to take up or release it (except in the presence of the Ca²⁺ ionophore A23187) (Prentki *et al.*, 1984a,b). Lastly, and perhaps most importantly, this steady state displays characteristics identical to those of a microsomal fraction derived from a similar insulin-secreting tissue (Prentki *et al.*, 1984a). Thus, the steady state maintained by isolated microsomes was quantitatively identical to that seen here, and was similarly dependent upon ATP. Although the microsomal fraction was crude, further purification showed clearly that the ATP-dependent Ca²⁺ uptake correlated with endoplasmic reticulum markers, and not at all with those of either the plasma membrane, mitochondria or secretory granules (Prentki *et al.*, 1984a).

The Ca²⁺ release in response to IP₃ was much larger if the cells had been allowed to pump down to the lower Ca²⁺ steady state, rather than if IP₃ was added at the mitochondrial set point. Indeed, the small release seen at the higher steady state is probably also derived from the ATP-dependent pool (see the Results section). These and the above data all suggest that IP₃ releases Ca²⁺ specifically from the endoplasmic reticulum, a conclusion consistent with the results of other investigators using permeabilized preparations of pancreatic acinar cells and hepatocytes (Streb *et al.*, 1983; Burgess *et al.*, 1984; Joseph *et al.*, 1984). Moreover, the results reported here confirm those obtained with the subcellular fractions of insulinoma tissue, in which it was shown that IP₃ released Ca²⁺ from the microsomal fraction, but not from mitochondria or secretory granules (Prentki *et al.*, 1984a).

The concentration-dependence of the IP₃ effect is very similar to that previously described for the exocrine pancreas (Streb *et al.*, 1983). However, in response to a maximal challenge the RINm5F cells released approximately twice as much Ca²⁺ (expressed in nmol of Ca²⁺/mg of protein) as did the acinar cells. The liver may be slightly more sensitive to IP₃, with an apparent K_m of 0.1 μM (Joseph *et al.*, 1984). However, points for the dose-response curve in that study were apparently assembled by sequential pulse additions of IP₃. Any slight attenuation of the subsequent re-

sponses, as seen here, would thus shift the curve to the left. At present, accurate measurements of the physiological concentrations of IP_3 are unavailable for comparison with the Ca^{2+} -release data. However, by using hepatocytes labelled with *myo*- $[^3H]$ -inositol, it has been calculated that the agonist-stimulated increase in IP_3 is more than sufficient to account for the observed Ca^{2+} mobilization (Burgess *et al.*, 1984).

The amount of Ca^{2+} released by sequential additions of IP_3 was time-dependent. With a 20 min interval between pulses, the release peaks were similar, but if the second pulse was added earlier the response was slightly inhibited. This can be compared with the degradation data because both experiments were performed under identical conditions and with similar concentrations of cells. These latter data suggest that most of the added $2.5 \mu M$ - IP_3 must be degraded before complete resensitization is restored to the system. This is consistent with the observations that insulinoma microsomes, which do not appreciably metabolize IP_3 , are completely insensitive to second and subsequent IP_3 additions (Prentki *et al.*, 1984a).

It has recently been suggested that IP_3 degradation is associated with the reuptake of Ca^{2+} after the initial release, although this was not the case for supramaximal IP_3 concentrations (Joseph *et al.*, 1984). Although reuptake and resensitization could be related, another explanation of the Ca^{2+} reuptake is possible. This applies especially to studies with subcellular fractions in which there may be heterogeneity of the microsomal Ca^{2+} compartments, some of which might lack IP_3 'receptors'. It may be that upon the addition of IP_3 , Ca^{2+} is released from the sensitive pool and, in the continued presence of IP_3 , could only be taken up by the insensitive pool where it would be then unavailable for subsequent release. Some experimental evidence for this has been obtained with a rat liver microsomal preparation (Dawson & Irvine, 1984). Such subpopulations of Ca^{2+} pools are conceptually more difficult to envisage in permeabilized cell preparations, but on the other hand the attenuation seen is only small compared with that in microsomes, and the degradation of IP_3 would allow Ca^{2+} to be transferred back to the releasable pool. In either case it could be concluded that the ultimate resensitization of the system is dependent upon breakdown of the IP_3 .

The stimulation of insulin release by nutrient carbohydrates is closely associated with a rise in the cytosolic Ca^{2+} concentration. Although Ca^{2+} influx from the extracellular space also plays an important role, available evidence suggests that mobilization of intracellular Ca^{2+} occurs as an early event during this response (Wollheim & Sharp, 1981; Wollheim & Pozzan, 1984). Other

early events promoted by glucose in insulin secretory tissue are the activation of PIP_2 hydrolysis (within 30 s of addition) (Laychock, 1983), and the stimulation of phospholipid synthesis *de novo* (within 1 min) (Di Marco *et al.*, 1984). Moreover, we have recently demonstrated that the nutrient secretagogue D-glyceraldehyde significantly raises IP_3 concentrations within 1 min of addition to RINm5F cells (Biden *et al.*, 1984). It is therefore apparent that IP_3 might link phospholipid turnover and Ca^{2+} mobilization, not only in response to receptor-mediated events, but in processes such as nutrient-stimulated insulin release that are not receptor-mediated. Such a conclusion is supported by the current demonstration that IP_3 mobilizes intracellular Ca^{2+} from permeabilized insulin secreting cells. Moreover, it acts specifically upon that pool (most probably the endoplasmic reticulum) which maintains a Ca^{2+} steady state at a level similar to the one previously described for intact RINm5F cells. IP_3 could thus act as the long-sought messenger linking the metabolic and cationic events associated with nutrient-stimulated insulin release.

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