myo-Inositol 1,4,5-Trisphosphate

A SECOND MESSENGER FOR THE HORMONAL MOBILIZATION OF INTRACELLULAR Ca\textsuperscript{2+} IN LIVER*

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It is well established that a redistribution of cellular Ca\textsuperscript{2+} is responsible for the physiological actions of many hormone and neurotransmitters that exert their binding to specific receptors located at the surface of the cell. In the liver, the glycolytic action of \(\alpha\)-adrenergic agonists and vasoactive peptide hormones is mediated by an increase in the cytosolic free Ca\textsuperscript{2+} concentration through allosteric activation of phosphorylase \(\beta\) kinase. This process

The stimulation of hepatocytes by \(\alpha\)-adrenergic agonists and vasoactive peptides results in a mobilization of intracellular Ca\textsuperscript{2+} which is accompanied by breakdown of phosphatidylinositol 4,5-bisphosphate to release myo-inositol 1,4,5-trisphosphate (Ins(1,4,5)P\textsubscript{3}). The possible involvement of Ins(1,4,5)P\textsubscript{3} in intracellular Ca\textsuperscript{2+} mobilization was tested using a preparation of saponin-permeabilized hepatocytes. Added Ca\textsuperscript{2+} was sequestered by intracellular organelles in the presence of ATP until the medium free Ca\textsuperscript{2+} concentration was lowered to a new steady state level. The subsequent addition of Ins(1,4,5)P\textsubscript{3} caused a rapid Ca\textsuperscript{2+} release, which was complete within 5 s. Half-maximal and maximal Ca\textsuperscript{2+} release were obtained at concentrations of Ins(1,4,5)P\textsubscript{3} of 0.1 and 0.5 \(\mu\)M, respectively. The maximal amount of Ca\textsuperscript{2+} mobilized was 450 pmol/mg of cell dry weight. Using experimental conditions designed to permit selective Ca\textsuperscript{2+} accumulation into mitochondrial or non-mitochondrial stores, it was determined that all of the Ca\textsuperscript{2+} released by Ins(1,4,5)P\textsubscript{3} originated from non-mitochondrial, vesicular stores.

After Ca\textsuperscript{2+} release was completed, reaccumulation occurred until the medium free Ca\textsuperscript{2+} concentration was restored to its original level. Experiments using \(^{32}\)P-labeled Ins(1,4,5)P\textsubscript{3} indicated that Ca\textsuperscript{2+} reaccumulation was associated with dephosphorylation of this compound. From a consideration of the properties of the Ca\textsuperscript{2+} release induced by Ins(1,4,5)P\textsubscript{3}, with respect to its kinetics, dose response, specificity, and the amount of Ca\textsuperscript{2+} released, the data strongly suggest that this compound is a second messenger involved in the hormonal mobilization of Ca\textsuperscript{2+} from intracellular stores.

In phosphorylase \(\beta\) activity can be observed in the complete absence of extracellular Ca\textsuperscript{2+}, indicating that the initial increase in cytosolic free Ca\textsuperscript{2+} is due to the mobilization of Ca\textsuperscript{2+} from intracellular compartments (3, 4). Although the identity of these compartments remains controversial, evidence has been presented to suggest that both mitochondrial and non-mitochondrial compartments may be involved (4–6). It is still not known how a hormone, which binds at the cell surface, can release Ca\textsuperscript{2+} from compartments located within the cell interior.

An increased hydrolysis of inositol phospholipids is associated with the action of calcium-mobilizing stimuli in a number of different cells (for reviews see Refs. 7 and 8). In the liver, such stimuli lead to the breakdown of PtdIns(4,5)P\textsubscript{2} by a phosphodiesterase with the release of Ins(1,4,5)P\textsubscript{3} and diacylglycerol (9–11). This breakdown of PtdIns(4,5)P\textsubscript{2} is not secondary to the elevation of cytosolic Ca\textsuperscript{2+} and occurs sufficiently rapidly to play a role in generating a messenger for mobilizing intracellular Ca\textsuperscript{2+} (9, 10). Since pathways exist for the rapid production and degradation of Ins(1,4,5)P\textsubscript{3} in liver and this compound is water-soluble, it appears to be the most promising intermediate of hormone-stimulated inositol lipid metabolism potentially capable of acting as a messenger for the mobilization of calcium from intracellular compartments. We have tested this hypothesis and data are presented in this communication to show that low concentrations of Ins(1,4,5)P\textsubscript{3} can cause a rapid release of Ca\textsuperscript{2+} from a non-mitochondrial store in saponin-permeabilized rat hepatocytes. Our data confirm and extend similar observations that have been made recently on the action of Ins(1,4,5)P\textsubscript{3} in a preparation of "leaky" pancreatic acinar cells (12) and in saponin-permeabilized guinea pig hepatocytes.

MATERIALS AND METHODS

Isolated hepatocytes were prepared as described previously (4) and, after two washes, were stored on ice in Ca\textsuperscript{2+}-free Krebs-Henseleit buffer. When Ca\textsuperscript{2+} movements were measured with a Ca\textsuperscript{2+}-sensitive

1 The abbreviations used are: Ins(1,4,5)P\textsubscript{3}, L-myoinositol 1,4,5-trisphosphate; PtdIns(4,5)P\textsubscript{2}, 1-(3-m-phosphatidyl)-L-myoinositol 1,4,5-bisphosphate; Ins(1,4,5)P\textsubscript{3}, L-myoinositol 1,4,5-bisphosphate; 1799, bis(hexafluoracetonyl)-amino-5-methylphenoxy)-methyl)-6-methoxy-3-his-(carboxymethyl)-aminoquinoline tetrakis-(acetoxymethyl)ester); pCa, minus log of free calcium.

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where The endogenous inositol lipid phosphodiesterase (phospholipase C) presumably reflects an equal distribution of 3'P between the 4 and prepared in a similar manner from erythrocyte ghosts after prelabeling the Quin 2 buffer. Calibration of the Quin 2-Ca fluorescence signal was made by adding 2.5 mM EGTA (plus Tris base) plus oligomycin (5 nM/ml) and rotenone (0.5 nM/ml). Substrates such as succinate (5 mM) and MgATP plus a creatine phosphate ATP regenerating system were added as required after saponin permeabilization was complete. Quin 2-Ca fluorescence was measured using a fluorometer (Johnson Research Foundation, Electronics Shop) with excitation at 385 nm and emission at 490–570 nm selected by specific filters. The cell suspension was continuously stirred in a cuvette chamber maintained at 37 °C. The required free Ca2+ in the medium was obtained by adding known amounts of Ca2+ to the Quin 2 buffer. Calibration of the Quin 2-Ca fluorescence signal was made by adding 2.5 mM EGTA (plus Tris base to give pH 8.3) followed by 5 mM CaCl2, essentially as described by Tsien et al. (18). Unlabeled Ins(1,4,5)P3 was prepared by hydrolysis of the naturally occurring PtdIns(4,5)P2 in erythrocyte ghosts or ox brain extracts. The endogenous inositol lipid phosphodiesterase (phospholipase C) activity of these tissues was stimulated by addition of 2 mM CaCl2 and the related Ins(1,4,5)P3 was separated by anion exchange chromatography. The details of the preparative procedure and authentication of Ins(1,4,5)P3 were essentially the same as those given elsewhere (17, 18). The concentration of Ins(1,4,5)P3 was determined by measurement of organic phosphorus (19). 32P-labeled Ins(1,4,5)P3 was prepared in a similar manner from erythrocyte ghosts after prelabeling the erythrocytes with 32Pi, for 12 h at 30°C. Under these conditions a constant specific activity of PtdIns(4,5)P2 was obtained which presumably reflects an equal distribution of 32P between the 4 and 5 positions on the inositol ring. Most other chemicals and biochemicals were obtained from the sources given previously (4, 9).

RESULTS AND DISCUSSION

Ins(1,4,5)P3 Specific Ca2+ Release from Permeabilized Liver Cells—In order to examine possible effects of Ins(1,4,5)P3 on the release of Ca2+ from intracellular pools, a preparation of hepatocytes was used in which the plasma membrane was permeabilized by saponin treatment. For measurement of Ca2+ movements using a Ca2+-sensitive electrode, isolated liver cells were incubated in a medium designed to mimic the ionic composition of cytosol containing high K+, low Na+, and no added Ca2+ (giving a free Ca2+ of 1–2 μM). When ATP was present in the medium prior to disruption of the plasma membrane by saponin, the Ca2+ concentration of the medium was reduced by sequestration into the newly exposed intracellular Ca2+ pools. After approximately 5 min, a steady state free Ca2+ of 0.45 μM (pCa2+ = 6.35) was established as measured with the Ca2+ electrode. Addition of Ins(1,4,5)P3 under these conditions resulted in a rapid release of Ca2+ which was then slowly reaccumulated until the original steady state free Ca2+ was restored (Fig. 1a). No Ca2+ release was observed with Ins(4,5)P2, l-myoinositol 2-phosphate, or fructose 2,6-bisphosphate. No significant response of the electrode was observed when Ins(1,4,5)P3 was added to the incubation buffer in the absence of hepatocytes (Fig. 1b).

Sequential additions of Ins(1,4,5)P3 produced cycles of Ca2+ release and reuptake in which the amount of Ca2+ released was dependent on the concentration of added Ins(1,4,5)P3. The large difference in electrode response upon Ca2+ additions in the presence and absence of permeabilized hepatocytes indicates considerable buffering by cellular Ca2+ binding sites (Fig. 1). Consequently, the amount of Ca2+ released by Ins(1,4,5)P3 was quantitated by making appropriate additions of known amounts of Ca2+ into the electrode chamber at the end of each trace.

The relationship between the concentration of Ins(1,4,5)P3 added and the amount of Ca2+ released is shown in Fig. 2. A maximum value of about 450 pmol of calcium/mg of cell dry weight was released by Ins(1,4,5)P3. This value is within the range of values reported for the amounts of Ca2+ released from intracellular stores in the perfused liver and isolated hepatocytes by concentrations of vasoactive peptides and α1-adrenergic agonists giving a maximum glycogenolytic response (3, 4, 20). Half-maximal and maximal amounts of Ca2+ release were obtained at Ins(1,4,5)P3 concentrations of 0.1 and 0.5 μM, respectively. These concentrations compare very well with our recent measurements of Ins(1,4,5)P3 in intact hepatocytes after vasopressin treatment. In that study, the increase of Ins(1,4,5)P3 which coincided with peak cytosolic free Ca2+ elevation was calculated to be 0.6 μM. Thus, both the amount of Ca2+ released by Ins(1,4,5)P3 and the concentration dependence of this release observed in the permeabilized hepatocyte are compatible with a role for Ins(1,4,5)P3 as

FIG. 1. Ca2+ release induced by Ins(1,4,5)P3 in saponin-permeabilized hepatocytes. Isolated hepatocytes (17 mg dry weight/ml) were incubated in the basic incubation medium (see "Materials and Methods") supplemented with 20 mM creatine phosphate and 8 unit/ml of creatine kinase. Saponin (15 μg/mg of cell dry weight) was added and the changes in medium free Ca2+ concentration were measured using a Ca2+ electrode. Trace a shows the effect of sequential additions of increasing concentrations of Ins(1,4,5)P3 (IP3). Trace b shows the effect of adding 1 μM Ins(1,4,5)P3 to the incubation medium in the absence of hepatocytes. The amounts of Ca2+ added (nanomole) for calibration are indicated in the figure.

FIG. 2. The relationship between the amount of Ca2+ released and the concentration of added Ins(1,4,5)P3. The amount of Ca2+ released in response to various concentrations of added Ins(1,4,5)P3 was determined from experiments similar to that shown in Fig. 1 (trace a). Where standard errors are shown, the results are the mean ± S.E. of 3–6 observations on 3 different cell preparations. The remaining points are the mean of duplicate determinations.
an intracellular messenger linking cell surface receptors to the mobilization of intracellular Ca\(^{2+}\) stores.

**Identification of the Pool of Ca\(^{2+}\) Released by Ins(1,4,5)P\(_3\)---**

The nature of the Ca\(^{2+}\) pool from which Ins(1,4,5)P\(_3\) releases Ca\(^{2+}\) in the saponin-permeabilized hepatocytes has been further investigated by using the fluorescent Ca\(^{2+}\) indicator Quin 2 as both a Ca\(^{2+}\) buffer and a Ca\(^{2+}\) indicator. This approach has some advantages over the Ca\(^{2+}\) electrode in that it allows the medium Ca\(^{2+}\) to be buffered at a fixed, predetermined concentration. In addition, the fluorimeter used for these studies has a time resolution of about 0.2 s (based on Ca\(^{2+}\) pulse additions) which allows resolution of the very rapid kinetics of Ins(1,4,5)P\(_3\)-induced Ca\(^{2+}\) release (see below). For experiments using Quin 2, hepatocytes were permeabilized with saponin in the K\(^+\) based buffer described above but with the addition of 75 \(\mu\)M Quin 2, which reduced the concentration of free Ca\(^{2+}\) in the medium to about 20 nM. Under these conditions, in the absence of added substances and in the presence of rotenone and oligomycin to prevent endogenous substrate formation and utilization, there was no uptake of medium Ca\(^{2+}\) during saponin treatment, and the endogenous Ca\(^{2+}\) content of intracellular organelles was minimal. When cell permeabilization was complete, Ca\(^{2+}\) could be added to give the required starting free Ca\(^{2+}\) concentration.

As shown in Fig. 3, when succinate was added to energize the mitochondrion of saponin-permeabilized hepatocytes, there was no significant uptake of Ca\(^{2+}\) if the medium free Ca\(^{2+}\) was in the range of 150–200 nM, which is believed to be the basal free Ca\(^{2+}\) in the cytosol of intact hepatocytes (21, 22). Addition of MgATP, however, caused a substantial sequestration of Ca\(^{2+}\) which amounted to about 2 nmol/mg of cell dry weight. Dissipation of the mitochondrial electrochemical proton gradient with the uncoupler, 1799 (23), caused only a small amount of Ca\(^{2+}\) release after ATP-dependent Ca\(^{2+}\) uptake, while essentially all of the sequestered Ca\(^{2+}\) could be released by the subsequent addition of the Ca\(^{2+}\) ionophore ionomycin (Fig. 3B). In the absence of ATP, the ionomycin-sensitive Ca\(^{2+}\) pool was negligible (Fig. 3C). These results are similar to those obtained in other permeabilized cell preparations (24, 25) where it has been shown that at Ca\(^{2+}\) concentrations believed to occur in the cytosol of unstimulated cells, the major intracellular site of Ca\(^{2+}\) sequestration is a non-mitochondrial, ATP-dependent calcium pool. The finding that this pool of Ca\(^{2+}\) can be released by ionomycin indicates that it is a vesicular pool, presumably associated with the reticular system of the cells.

In agreement with the results obtained using the Ca\(^{2+}\) electrode (Fig. 1), addition of Ins(1,4,5)P\(_3\) after ATP-dependent Ca\(^{2+}\) uptake into the saponized hepatocytes resulted in a rapid Ca\(^{2+}\) release followed by a slower reaccumulation of this Ca\(^{2+}\) (Fig. 3A). Ins(1,4,5)P\(_3\) did not release Ca\(^{2+}\) after addition of ionomycin (Fig. 3B) or in the absence of ATP (Fig. 3C) and there was no alteration in the distribution of Ca\(^{2+}\) between the mitochondrial and non-mitochondrial pools after Ins(1,4,5)P\(_3\) treatment (Fig. 3, A versus B). These data indicate that Ins(1,4,5)P\(_3\) releases Ca\(^{2+}\) from a vesicular ATP-dependent, non-mitochondrial pool while reuptake of Ca\(^{2+}\) occurs into the same or a similar pool. Our finding that multiple cycles of Ca\(^{2+}\) release and reuptake can be obtained even after addition of a saturating concentration of Ins(1,4,5)P\(_3\) (Fig. 1) also suggests that the Ins(1,4,5)P\(_3\)-sensitive Ca\(^{2+}\) pool refills during the reuptake phase.

Using both cell fractionation and nondisruptive techniques, it has previously been found that mitochondria contain substantial amounts of Ca\(^{2+}\) in the intact hepatocyte and that, in addition to the non-mitochondrial calcium pool that can be

**FIG. 3. Characterization of the Ins(1,4,5)P\(_3\)-sensitive Ca\(^{2+}\) pool.** Isolated hepatocytes were incubated at 5 mg of cell dry weight/ml in the standard K\(^+\) based medium supplemented with 75 \(\mu\)M Quin 2, 3 \(\mu\)g/ml oligomycin, 0.5 \(\mu\)g/ml of rotenone, and 75 \(\mu\)M of saponin. After cell permeabilization was complete (about 5 min), CaCl\(_2\) was added to give the required free Ca\(^{2+}\) concentration. Substrates were then added as indicated: Succ, 5 mM succinate; MgATP, 3 mM MgATP precluded by addition of 10 mM creatine phosphate and 10 units/ml of creatine kinase. Changes of the medium free Ca\(^{2+}\) concentration were followed by measuring the fluorescence of the Quin 2-Ca complex as outlined under "Materials and Methods." At steady state, 0.5 \(\mu\)M Ins(1,4,5)P\(_3\) (IP\(_3\)) was added and the magnitude of Ins(1,4,5)P\(_3\)-induced Ca\(^{2+}\) release was estimated from a subsequent calibration addition of 2 nmol/ml of CaCl\(_2\) (Ca\(^{2+}\)). The distribution of Ca\(^{2+}\) between mitochondrial and non-mitochondrial vesicular Ca\(^{2+}\) pools was determined by sequential additions of 5 \(\mu\)M uncoupler 1799 followed by 5 \(\mu\)g/ml of ionomycin. In trace A, Ins(1,4,5)P\(_3\) was added after substrate-induced Ca\(^{2+}\) sequestration, in trace B, vesicular Ca\(^{2+}\) pools were released by ionophores before Ins(1,4,5)P\(_3\) addition, and in trace C, ATP was omitted.

mobilized by hormones, Ca\(^{2+}\) can also be released from the mitochondrial pool after hormone treatment (4, 5). Since the mitochondria of the saponin-permeabilized hepatocytes do not take up Ca\(^{2+}\) under the conditions used in Fig. 3, it was not possible to determine whether Ins(1,4,5)P\(_3\) can also release Ca\(^{2+}\) from the mitochondrial Ca\(^{2+}\) pool. Under these conditions, the discrepancy in mitochondrial calcium content between the saponin-permeabilized hepatocyte and our previous measurements with intact hepatocytes could result from the dilution of some cytosolic effector of mitochondrial Ca\(^{2+}\) transport. This and other possibilities are currently under investigation.

As shown in Figs. 1a and 3A, in saponin-treated cells which had taken up Ca\(^{2+}\) to a steady state in the presence of ATP, subsequent addition of CaCl\(_2\) did not result in further Ca\(^{2+}\) uptake. This indicates that the ATP-dependent Ca\(^{2+}\) pool was saturated. However, after further additions of Ca\(^{2+}\) had elevated the free Ca\(^{2+}\) to greater than 0.7 \(\mu\)M, a second lower affinity Ca\(^{2+}\) pool was revealed and the added Ca\(^{2+}\) was again sequestered (Fig. 1a). The ability of this second pool to completely remove sequential pulses of Ca\(^{2+}\) and maintain a constant steady state free Ca\(^{2+}\) concentration in the medium
resembles the "set point" behavior of isolated mitochondria (26). Inhibition of Ca\(^{2+}\) uptake into this pool by ruthenium red, uncouplers, and respiratory chain inhibitors, suggests that the lower affinity Ca\(^{2+}\) pool represents Ca\(^{2+}\) uptake by mitochondria. In order to assess possible effects of Ins\((1,4,5)\)P\(_3\) on mitochondrial Ca\(^{2+}\) release, the action of this compound was tested under conditions where the free Ca\(^{2+}\) of the medium was elevated to a level where appreciable amounts of Ca\(^{2+}\) were sequestered within the mitochondria.

In the experiment shown in Fig. 4A, after the completion of ATP-dependent Ca\(^{2+}\) uptake, further Ca\(^{2+}\) was added and the mitochondria were allowed to accumulate 2–3 nmol of Ca\(^{2+}\)/mg of cell dry weight (equivalent to about 10 nmol of Ca\(^{2+}\)/mg of mitochondrial protein). The addition of a small Ca\(^{2+}\) pulse clearly shows that the mitochondria in these cells maintained the medium free Ca\(^{2+}\) at approximately 0.7 \(\mu\)M. Under these conditions, Ins\((1,4,5)\)P\(_3\) was able to release a similar amount of Ca\(^{2+}\) to that observed at the lower medium free Ca\(^{2+}\) used in Figs. 1–3. However, all of this Ca\(^{2+}\) was apparently still derived from the non-mitochondrial pool because no Ins\((1,4,5)\)P\(_3\)-induced Ca\(^{2+}\) release was observed in the absence of ATP (Fig. 4B). Furthermore, the Ca\(^{2+}\) release observed when mitochondrial Ca\(^{2+}\) uptake was blocked by the addition of ruthenium red and omission of succinate (Fig. 4C) was identical to that when the mitochondria did contain Ca\(^{2+}\) (Fig. 4A). Thus, the data presented above clearly show that Ins\((1,4,5)\)P\(_3\) does not release Ca\(^{2+}\) from the mitochondrial pool in permeabilized hepatocytes and that the observed Ca\(^{2+}\) release originates entirely from a non-mitochondrial, vesicular compartment.

**Fig. 5. Degradation of \(^{32}\)P-Ins\((1,4,5)\)P\(_3\) in saponin-permeabilized hepatocytes.** The non-mitochondrial Ca\(^{2+}\) pool of saponin-permeabilized hepatocytes was loaded with Ca\(^{2+}\) in the presence of MgATP exactly as described for Fig. 3, trace A. At steady state, 1 \(\mu\)M \(^{32}\)P-labeled Ins\((1,4,5)\)P\(_3\) (IP\(_3\)) was added and the resulting Ca\(^{2+}\) release and subsequent reuptake was measured by using Quin 2-Ca fluorescence. The kinetics of Ca\(^{2+}\) release were resolved in this experiment by using a fast chart speed during the first 10 s after Ins\((1,4,5)\)P\(_3\) addition. Ins\((1,4,5)\)P\(_3\) breakdown was measured in a parallel incubation where samples (250 \(\mu\)l) were removed at the times indicated and quenched in 250 \(\mu\)l of 20% trichloroacetic acid. For the zero time point, \(^{32}\)P-Ins\((1,4,5)\)P\(_3\) was added into a trichloroacetic acid extract. The deprotonized samples were neutralized and diluted to 2 ml before separation of inositol phosphates on Dowex 1-X8 anion exchange columns as described by Berridge et al. (18). Each point is the mean of values from two separate incubations.

Significance of the Ca\(^{2+}\) Reuptake Response—In all cases where Ca\(^{2+}\) release after Ins\((1,4,5)\)P\(_3\) treatment has been observed, at least part of the released Ca\(^{2+}\) was always reaccumulated within the following 2–5 min. The transient nature of the Ins\((1,4,5)\)P\(_3\)-induced Ca\(^{2+}\) release could result from some intrinsic autoregulation of the Ca\(^{2+}\) release mechanism. However, Fig. 1a shows that multiple cycles of Ca\(^{2+}\) release and reuptake can be obtained with sequential Ins\((1,4,5)\)P\(_3\) additions. Taken together with the observation that the time taken for Ca\(^{2+}\) reuptake increases with increasing Ins\((1,4,5)\)P\(_3\) concentrations beyond that required for maximal Ca\(^{2+}\) release (Fig. 1a), these results suggest that the reuptake phase might be the result of degradation of Ins\((1,4,5)\)P\(_3\). In order to investigate this possibility, \(^{32}\)P-labeled Ins\((1,4,5)\)P\(_3\) was used to measure its rate of disappearance during the Ca\(^{2+}\) release-reuptake cycle. Fig. 5 shows the Ca\(^{2+}\) released induced by \(^{32}\)P-labeled Ins\((1,4,5)\)P\(_3\) as measured using Quin 2, under conditions similar to those used in Fig. 3A. The time scale has been expanded to show the rapidity of the Ca\(^{2+}\) release, which is complete within 5 s and occurs at an initial rate of about 250 pmol/mg of cell dry weight·s\(^{-1}\). The amount of \(^{32}\)P-Ins\((1,4,5)\)P\(_3\) remaining at various times is shown superimposed on the Quin 2-Ca trace. It is clear from Fig. 5 that Ins\((1,4,5)\)P\(_3\) degradation coincides with the reuptake of Ca\(^{2+}\). Although degradation of the messenger partially explains the transient nature of Ins\((1,4,5)\)P\(_3\)-induced Ca\(^{2+}\) release, other factors are probably also involved since the ability of increasing Ins\((1,4,5)\)P\(_3\) concentrations to delay Ca\(^{2+}\) reuptake is rather limited. It was found that the half-time for reuptake of released Ca\(^{2+}\) did not increase further when concentrations of Ins\((1,4,5)\)P\(_3\) in excess of 6 \(\mu\)M were used (data not shown).

Ins\((1,4,5)\)P\(_3\) did not release Ca\(^{2+}\) from purified microsomal vesicles, derived from a high speed spin (80,000 x g) of a postmitochondrial supernatant (27), and also did not release Ca\(^{2+}\) from purified plasma membrane vesicles or mitochondria.

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K. E. Coll, S. K. Joseph, and J. R. Williamson, unpublished observations.
(data not shown). However, if Ins(1,4,5)P3 is added to various subcellular fractions of rat liver, Ca\textsuperscript{2+} release can be obtained from a crude mitochondrial fraction that is heavily contaminated by endoplasmic reticulum and which is obtained at much lower centrifugal forces than used to prepare microsomal vesicles. The exact nature of this fraction and the mechanisms of Ins(1,4,5)P3-induced Ca\textsuperscript{2+} release is currently under investigation.

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Cell-free translation of calf type III collagen. Effect of magnesium on ribosome movement during elongation.

Louis Gerstenfeld, John C. Beldekas, Carl Franzblau, and Gail E. Sonenshein

Page 12060, Fig. 2B. The designations of type I and type III were omitted. The corrected figure is shown below:

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myo-Inositol 1,4,5-trisphosphate. A second messenger for the hormonal mobilization of intracellular Ca²⁺ in liver.

Suresh K. Joseph, Andrew P. Thomas, Rebecca J. Williams, Robin F. Irvine, and John R. Williamson

It has been brought to the authors' attention that there are a number of nomenclature errors in this paper. The stereochemistry of the inositol ring and a correction of the initial errors of nomenclature made by the Commission on Biochemical Nomenclature are discussed by Bernard W. Agranoff in TIBS (1978) 3, N283–N285. In brief, in inositol phospholipids, the natural compounds are in the D, rather than L, series.

Footnote 1: The first three lines should read:

The abbreviations used are: Ins(1,4,5)P₃, D-myoinositol-1,4,5-trisphosphate; PtdIns(4,5)P₂, 1-(3-sn-phosphatidyl)-D-myoinositol 4,5-bisphosphate; Ins(1,4)P₂, D-myoinositol 1,4-bisphosphate.

Page 3078, tenth line from end

Ins(4,5)P₂ should read Ins(1,4)P₂ and L-myoinositol 2-phosphate should read myoinositol 2-phosphate.

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.