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Metabolism of inositol 1,4,5-trisphosphate in guinea-pig hepatocytes

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Metabolism of inositol 1,4,5-trisphosphate was investigated in permeabilized guinea-pig hepatocytes. The conversion of [^{3}H]inositol 1,4,5-trisphosphate to a more polar ^{3}H-labelled compound occurred rapidly and was detected as early as 5 s. This material co-eluted from h.p.l.c. with inositol 1,3,4,5 tetrakis[^{32}P]phosphate and is presumably an inositol tetrakisphosphate. A significant increase in the ^{3}H-labelled material co-eluting from h.p.l.c. with inositol 1,3,4-trisphosphate occurred only after a definite lag period. Incubation of permeabilized hepatocytes with inositol 1,3,4,5-tetrakis[^{32}P]phosphate resulted in the formation of ^{32}P-labelled material that co-eluted with inositol 1,3,4-trisphosphate; no inositol 1,4,5-tris[^{32}P]phosphate was produced, suggesting the action of a 5-phosphomonoesterase. The half-time of hydrolysis of inositol 1,3,4,5-tetrakis[^{32}P]phosphate of approx. 1 min was increased to 3 min by 2,3-bisphosphoglyceric acid. Similarly, the rate of production of material tentatively designed as inositol 1,3,4-tris[^{32}P]phosphate from the tetrakisphosphate was reduced by 10 mM-2,3-bisphosphoglyceric acid. In the absence of ATP there was no conversion of [^{3}H]inositol 1,4,5-trisphosphate to [^{3}H]inositol tetrakisphosphate or to [^{3}H]inositol 1,3,4-trisphosphate, which suggests that the 1,3,4 isomer does not result from isomerization of inositol 1,4,5-trisphosphate. The results of this study suggest that the origin of the 1,3,4 isomer of inositol trisphosphate in isolated hepatocytes is inositol 1,3,4,5-tetrakisphosphate and that inositol 1,4,5-trisphosphate is rapidly converted to this tetrakisphosphate. The ability of 2,3-bisphosphoglyceric acid, an inhibitor of 5-phosphomonoesterase of red blood cell membrane, to inhibit the breakdown of the tetrakisphosphate suggests that the enzyme which removes the 5-phosphate from inositol 1,4,5-trisphosphate may also act to convert the tetrakisphosphate to inositol 1,3,4-trisphosphate. It is not known if the role of inositol 1,4,5-trisphosphate kinase is to inactivate inositol 1,4,5-trisphosphate or whether the tetrakisphosphate product may have a messenger function in the cell.

INTRODUCTION

Activation of hepatocytes by Ca^{2+}-mobilizing hormones is associated with the production of two isomeric forms of inositol trisphosphate: Ins(1,4,5)P_3 and Ins(1,3,4)P_3 (Burgess *et al.*, 1985). Studies by Irvine *et al.* (1984, 1985) initially defined the production of the two isomers of InsP_3 in rat parotid gland and subsequently these isomers have been described in hepatocytes, HL-60 leukaemia cells (Burgess *et al.*, 1985) and pancreatic acinar cells (Merritt *et al.*, 1986); however, the possible origin of the Ins(1,3,4)P_3 isomer and its functional role (if any) have been unknown. The production of Ins(1,4,5)P_3 is consistent with the phosphodiesteratic hydrolysis of PtdIns(4,5)P_2 but the production of Ins(1,3,4)P_3 cannot be accounted for by hydrolysis of this lipid, and the possible parent lipid, PtdIns(3,4)P_2, has not been identified in those cells which have been thus far examined (Irvine *et al.*, 1985). The recent identification of Ins(1,3,4,5)P_4 in rat cerebral cortical slices (Batty *et al.*, 1985), GH4 cells, Swiss 3T3 cells and blowfly salivary gland (Heslop *et al.*, 1985) and demonstration of the Ins(1,4,5)P_3 kinase activity in homogenates of rat brain, liver and pancreas (Irvine *et al.*, 1986) has provided the first possible answer to the question of the origin of the 1,3,4 isomer of InsP_3. That is, that Ins(1,3,4)P_3 might result from the sequential 3-phosphorylation of Ins(1,4,5)P_3 to Ins(1,3,4,5)P_4 which would be then dephosphorylated to Ins(1,3,4)P_3 (Irvine *et al.*, 1986). The present studies were carried out to determine whether such a mechanism could explain the accumulation of Ins(1,3,4)P_3 previously described in stimulated guinea-pig hepatocytes (Burgess *et al.*, 1985).

MATERIALS AND METHODS

Preparation of isolated intact and permeabilized hepatocytes

Isolated hepatocytes were prepared by collagenase digestion of the livers of male Hartley guinea pigs (Burgess *et al.*, 1981). The cells were permeabilized with saponin (75 μg/ml; Burgess *et al.*, 1983) in a cytosolic-type medium, which consisted of (mM): NaCl, 20.0; KCl, 100; $MgSO_4$, 5.0; NaH_2PO_4, 0.96; $NaHCO_3$, 25; EGTA, 1.0 and 2% (w/v) albumin at pH 7.2 and 37 °C for a period of 10 min. Following this treatment 99% of the cells were permeable to Trypan Blue. The cells were then

Abbreviations used: Ins$P$, inositol phosphate; InsP_2, inositol bisphosphate; Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; Ins(1,3,4)P_3, inositol 1,3,4,-trisphosphate; Ins(1,3,4,5)P_4, inositol 1,3,4,5-tetrakisphosphate; InsP_4, inositol tetrakisphosphate (phosphate positions unknown); 2,3-PGA, 2,3-bisphosphoglyceric acid; PtdIns(3,4)P_2, phosphatidylinositol 3,4-bisphosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate.

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washed and suspended in a medium which resembled that described above except that the free [Ca^{2+}] was buffered with EGTA to 180 nM (Burgess *et al.*, 1983). For all experiments except those examining the ATP-dependency of inositol phosphate production, ATP (1.5 mM) and an ATP-regenerating system consisting of creatine kinase (5 units/ml) and phosphocreatine (5 mM) were included in the incubation.

Formation of [^{3}H]InsP_4 in intact and permeable hepatocytes

Isolated guinea-pig hepatocytes were preincubated with [^{3}H]inositol to label inositol lipids as described by Burgess *et al.* (1985). Aliquots of the cell suspension were taken just prior to, and at various times after, the addition of 1 μM-angiotensin II to the cells. In the studies with permeable hepatocytes, the saponin-treated cells (25–30 mg of cell protein/ml) were incubated with 100 nM-[^{3}H]Ins(1,4,5)P_3 in the presence or absence of 10 mM-2,3-PGA for specified time periods. For both the intact and permeable cells, the reactions were terminated by the addition of 500 μl aliquots of cells to 1 ml of ice-cold 15% (w/v) trichloroacetic acid containing [^{32}P]Ins(1,4,5)P_3 and [^{32}P]Ins(1,3,4,5)P_4 (the preparation of which is described below) to act as markers for the radioactive peaks when the inositol phosphates in samples were separated by h.p.l.c. The samples were intermittently vortex-mixed and left on ice for 10 min, then centrifuged at 500 *g* for 10 min. The supernatant was removed and washed five times with 5 vol. of water-saturated diethyl ether to remove the trichloroacetic acid and then neutralized with NH_4OH (Irvine *et al.*, 1977).

Separation of inositol phosphates by h.p.l.c.

Inositol phosphates were separated by h.p.l.c. using a Whatman Partisil 10 SAX anion-exchange column and slight modifications of the ammonium formate/phosphate (pH 3.7) gradients described by Heslop *et al.* (1985) and Burgess *et al.* (1985). All inositol phosphates except Ins(1,3,4,5)P_4 were eluted by a non-linear gradient (convex, no. 4 on a Waters model 660 gradient programmer) at 1.2 ml/min increasing from 0 to 1 M-ammonium formate/phosphate for 25 min. The 1 M buffer was run for a further 5 min and the gradient was then immediately changed to a linear gradient increasing from 1 M- to 1.7 M-ammonium formate/phosphate in 3 min at 1.2 ml/min. This final solution was then continued at 1.2 ml/min for a further 23 min. [^{32}P]Ins(1,4,5)P_3 and [^{32}P]Ins(1,3,4,5)P_4 were added to the trichloroacetic acid prior to addition of the cell sample and so were present throughout the extraction process and could be used to identify tentatively the corresponding ^{3}H-labelled materials, and to calculate recoveries. [^{3}H]Ins(1,3,4)P_3 was tentatively identified as material eluting immediately prior to Ins(1,4,5)P_3, where in a previous study, material chemically identified as Ins(1,3,4)P_3 was shown to elute (Burgess *et al.*, 1985). In the Figures and in the discussions of the experimental findings of this study, the designations [^{3}H]Ins(1,4,5)P_3 in the intact cells, [^{3}H]Ins(1,3,4)P_3 in permeable cells, and [^{3}H]Ins(1,3,4,5)P_4 in intact and permeable cells are based on co-elution with chemically characterized material on h.p.l.c. rather than direct chemical analysis, and must be considered therefore as tentative assignments.

Preparation of [^{32}P]Ins(1,3,4,5)P_4

Preparation of [^{32}P]Ins(1,3,4,5)P_4 was according to the method described by Irvine *et al.* (1986). Rat brain homogenate (2%) in Tris-buffered sucrose (0.15 M-sucrose and 0.5 M-Tris/maleate, pH 7.5) was centrifuged at 1400 *g* for 10 min at 5 °C. A 500 μl aliquot of the supernatant was incubated with 500 nM-[^{32}P]Ins(1,4,5)P_3 (24 Ci/mmol) for 5 min in Tris-buffered sucrose containing 10 mM-ATP and 20 mM-$MgCl_2$ in a final volume of 2 ml. The reaction was terminated by addition of the sample to trichloroacetic acid (final concn. 14%) and centrifuged at 500 *g* for 10 min. The supernatant was removed, washed with diethyl ether and neutralized with NH_4OH as described above. The inositol phosphates in an aliquot of the sample were separated by h.p.l.c. to identify the extent of conversion of Ins(1,4,5)P_3 to Ins(1,3,4,5)P_4. Between 85 and 98% of Ins(1,4,5)P_3 was converted to Ins(1,3,4,5)P_4, the remaining radioactivity being in Ins$P$ and InsP_2, Ins(1,3,4)P_3 and Ins(1,4,5)P_3. The remainder of the sample was added to a Dowex (formate form) anion-exchange column. Each column was prepared by addition of 1 ml of a 50% (w/v) Dowex suspension to a glass pasteur pipette containing a glass wool plug as described by Irvine (1986). Inositol phosphates in each sample were separated using elution buffers A, B and C containing 0.1 M-formic acid and either 0.4 M- (A), 0.8 M- (B) or 1.0 M- (C) ammonium formate, respectively. The samples were eluted with 12 ml of buffer A to elute free inositol, Ins$P$ and InsP_2; 4 ml of buffer B to elute InsP_3 and 8 ml of buffer C to elute Ins(1,3,4,5)P_4. In our hands, this elution regime does not perfectly separate tris- and tetrakis-phosphates, since some Ins(1,3,4,5)P_4 is eluted with buffer B. However, this did not result in a problem with the brain homogenates since such a small proportion of the total radioactivity was trisphosphates. The eluent collected from elution with buffer C was run on a Dowex 50 column (H^+ form) to remove NH_4^+ ions, then frozen and lyophilized. The purity of the [^{32}P]Ins(1,3,4,5)P_4 was assayed by h.p.l.c. and found to range between 95 and 100%. The [^{32}P]Ins(1,3,4,5)P_4 used for experiments in which metabolism of Ins(1,3,4,5)P_4 was studied was greater than 99% pure, whereas the less pure material (95–99%) was used as a standard for h.p.l.c.

Analysis of the metabolism of [^{32}P]Ins(1,3,4,5)P_4 by permeabilized hepatocytes

Permeabilized hepatocytes were incubated with 100 nM-Ins(1,4,5)P_3 for 2 min, at which time [^{32}P]Ins(1,3,4,5)P_4 and 10 mM-2,3-PGA (or equivalent volume of buffer) were added to the incubation. The reaction was terminated at the specified times by addition of 500 μl aliquots of cells to trichloroacetic acid as described above. The trichloroacetic acid contained a standard of [^{3}H]Ins(1,4,5)P_3, and the inositol phosphates were separated by h.p.l.c. using the elution procedure described above.

Analysis of the ATP-dependency of Ins(1,4,5)P_3 metabolism

Permeabilized hepatocytes were incubated with 100 nM-[^{3}H]Ins(1,4,5)P_3 and 2,4-dinitrophenol (0.5 mM) in the presence or absence of ATP, phosphocreatine and creatine kinase for 2 min. Dinitrophenol was required to eliminate a slight apparent Ins(1,4,5)P_3 kinase activity

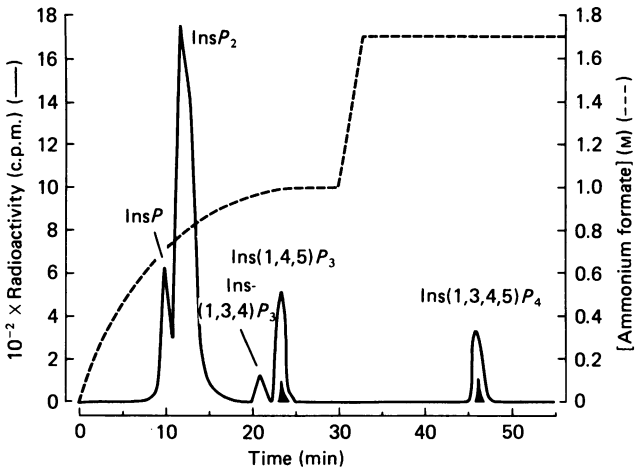

Fig. 1. Elution profile for separation of inositol phosphates by h.p.l.c.

Permeabilized hepatocytes were incubated with [^{3}H]Ins(1,4,5)P_3 for 5 min in the presence of 10 mM-2,3-PGA and prepared for analysis by h.p.l.c. as described in the Materials and methods section. The radioactive peaks for [^{3}H]inositol phosphates and for the ^{32}P-labelled standards (shaded peaks), [^{32}P]Ins(1,4,5)P_3 and [^{32}P]Ins(1,3,4,5)P_4, are represented in this typical elution profile; ---- indicates the ammonium formate concentration of the h.p.l.c. gradient.

which was detected in permeabilized hepatocytes incubated in the absence of ATP, phosphocreatine and creatine kinase. The reaction was terminated by addition of 500 μl of cells to 1 ml of 15% (w/v) trichloroacetic acid containing a [^{32}P]Ins(1,4,5)P_3 standard and inositol phosphates were separated by h.p.l.c. as described above.

Materials

Ins(1,4,5)P_3 and [^{3}H]Ins(1,4,5)P_3 (1 Ci/mmol) were obtained from Amersham, and [^{3}H]Ins(1,4)P_2 (2 Ci/mmol) and [^{32}P]Ins(1,4,5)P_3 (24 Ci/mmol) were obtained from NEN Research Products. ATP, phosphocreatine, creatine kinase and 2,3-PGA were obtained from Sigma. All other chemicals used were reagent grade.

RESULTS

Identification of radioactive peaks from h.p.l.c. analysis of samples

Fig. 1 shows a typical elution profile of a sample prepared from hepatocytes incubated with [^{3}H]Ins(1,4,5)P_3 as described in the Materials and methods section. The two initial peaks at 10 and 12 min probably represent inositol mono- and bis-phosphates, respectively, since they co-eluted with standard samples of these inositol phosphates on h.p.l.c. [The steepness of the convex gradient used in the region where Ins$P$ and InsP_2 elute did not always give baseline separation of the rather small quantities of Ins$P$ formed from the larger amounts of InsP_2. Thus, data for a combined InsP_2 and Ins$P$ fraction are reported, but in all cases this represents chiefly InsP_2.] [^{3}H]Ins(1,4,5)P_3 was tentatively identified by co-elution with [^{32}P]Ins(1,4,5)P_3 prepared from red cell membranes, as previously described (Burgess *et al.*, 1985) or obtained from New England Nuclear. The material immediately preceding Ins(1,4,5)P_3 in this system was identified by periodate degradation in a prior study as Ins(1,3,4)P_3 (Burgess *et al.*, 1985). The single peak of ^{3}H, which in this study eluted with 1.7 M-ammonium formate, co-eluted with standard [^{32}P]Ins-(1,3,4,5)P_4 prepared as described by Irvine *et al.* (1986). It seems likely that this material is [^{3}H]Ins(1,3,4,5)P_4, although we have not characterized it structurally.

[^{3}H]Inositol phosphates in intact hepatocytes

Fig. 2 shows the increase in [^{3}H]inositol phosphates designated as Ins(1,4,5)P_3, Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4 in the initial 30 s after activation with 1 μM-angiotensin II. As shown previously (Burgess *et al.*, 1985), [^{3}H]Ins(1,4,5)P_3 increased rapidly, followed after some delay by [^{3}H]Ins(1,3,4)P_3. The material designated as [^{3}H]Ins(1,3,4,5)P_3 also increased rapidly, but appeared to lag slightly behind the [^{3}H]Ins(1,4,5)P_3.

Metabolism of [^{3}H]Ins(1,4,5)P_3 in permeable hepatocytes

The incubation of permeabilized hepatocytes with [^{3}HIns(1,4,5)P_3 resulted in the formation of peaks of ^{3}H with h.p.l.c. mobilities associated with [^{3}H]Ins(1,3,4,5)P_4, Ins(1,3,4)P_3, InsP_2 and Ins$P$ (Figs. 3 and 4). Fig. 3 shows that [^{3}H]Ins(1,3,4,5)P_4 was detected as early as 5 s and continued to increase up to 2 min, followed by a decrease at 5 and 15 min. The production of [^{3}H]Ins(1,3,4)P_3 appeared to lag behind [^{3}H]Ins(1,3,4,5)P_4 formation and did not significantly increase above control until 1 min, after which time it continued to increase up to 5 min and then declined by 15 min. The delay in formation of this material suggests that it is a metabolite of Ins(1,3,4,5)P_4, which is consistent with the results of experiments in which [^{32}P]Ins(1,3,4,5)P_4 was added to permeabilized hepatocytes (discussed below). The major metabolite of [^{3}H]Ins(1,4,5)P_3 at any time point from 5 s to 15 min was in the InsP_2 region (and some in the Ins$P$ region). This could be accounted for by the action of [^{3}H]Ins(1,4,5)P_3 5-phosphatase which rapidly hydrolysed Ins(1,4,5)P_3 (Downes *et al.*, 1982; Seyfred *et al.*, 1984; Storey *et al.*, 1984) before it could be phosphorylated by an

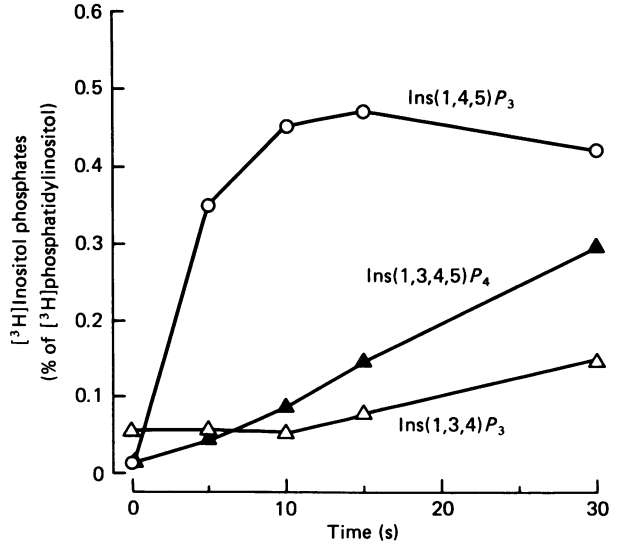

Fig. 2. Formation of inositol phosphates in angiotensin II-stimulated hepatocytes

Results of a single experiment are shown. Two other experiments gave similar results.

Ins(1,4,5)P_3 kinase. The 5-phosphatase inhibitor 2,3-PGA (Downes *et al.*, 1982), might be expected therefore to increase substrate availability for the kinase and to thereby increase the formation of InsP_4 and its metabolites.

As shown by comparison of Figs. 3 and 4, the rate of hydrolysis of Ins(1,4,5)P_3 and its conversion to less polar metabolites was reduced by 2,3-PGA. Fig. 5 shows the production of [^{3}H]Ins(1,3,4,5)P_4 and [^{3}H]Ins(1,3,4)P_3 during the first 5 min in these same experiments. The production of Ins(1,3,4,5)P_4 in the presence or absence of 2,3-PGA was comparable for the first 30 s, after which time there was a considerably greater formation of Ins(1,3,4,5)P_4 at 2 and 5 min with 2,3-PGA present. This suggested that either the increased availability of Ins(1,4,5)P_3 by inhibition of the Ins(1,4,5)P_3 5-phosphatase allowed greater production of Ins(1,3,4,5)P_4 or that the dephosphorylation of Ins(1,3,4,5)P_4 was reduced by 2,3-PGA. Since 2,3-PGA appeared to inhibit the production of both [^{3}H]InsP_2 (Figs. 3 and 4) and [^{3}H]Ins(1,3,4)P_3 (Fig. 5), it is likely that a combination of these actions occurred.

The results of the experiments examining the metabolism of [^{3}H]Ins(1,4,5)P_3 suggest that an Ins(1,4,5)P_3 kinase, perhaps similar to or identical with the 3-kinase described by Irvine *et al.* (1986), is present in guinea-pig hepatocytes. These results are also consistent with the idea that Ins(1,3,4)P_3 results from the hydrolysis of Ins(1,3,4,5)P_4 by a 5-phosphatase, which may be the same enzyme which acts on Ins(1,4,5)P_3. Further support for such a pathway in the guinea-pig hepatocyte was obtained from experiments examining the metabolism of [^{32}P]Ins(1,3,4,5)P_4.

Metabolism of [^{32}P]Ins(1,3,4,5)P_4

Since chemically pure Ins(1,3,4,5)P_4 was not available, the metabolism of [^{32}P]Ins(1,3,4,5)P_4 was studied in the permeable hepatocytes preincubated with Ins(1,4,5)P_3 for 2 min prior to the addition of tracer quantities of high specific radioactivity [^{32}P]Ins(1,3,4,5)P_4 (Fig. 6). Thus on the basis of the data in Fig. 5, the concentration of Ins(1,3,4,5)P_4 was assumed to be about 6 nM. Under these conditions, the [^{32}P]Ins(1,3,4,5)P_4 was rapidly converted to Ins(1,3,4)P_3; no Ins(1,4,5)P_3 was produced, which indicated that there is no detectable 3-phosphatase activity in this preparation. When 2,3-PGA was added with the [^{32}P]Ins(1,3,4,5)P_4, the rate of [^{32}P]Ins(1,3,4,5)P_4 hydrolysis was reduced and similarly, the production of [^{32}P]Ins(1,3,4)P_3 was slowed. The other metabolite of [^{32}P]Ins(1,3,4,5)P_4 is assumed to be [^{32}P]P_i, although this system does not resolve P_i from Ins$P$ well. No [^{32}P]InsP_2 was detected in these experiments. This could be due to one of several reasons: the Ins(1,3,4)P_3 could leak from the permeable cells more rapidly than it is metabolized; the Ins(1,3,4)P_3 could be metabolized to an InsP_2 which is very rapidly further degraded to an Ins$P$; or the Ins(1,3,4)P_3 could be metabolized to Ins(1,3)P_2, which would not be radiolabelled. The substantially greater amount of radioactivity in the presumed P_i fraction as compared with the Ins(1,3,4)P_3 fraction may indicate higher specific radioactivity in the 5-phosphate than in the 4-phosphate in the [^{32}P]Ins(1,4,5)P_3 obtained from New England Nuclear.

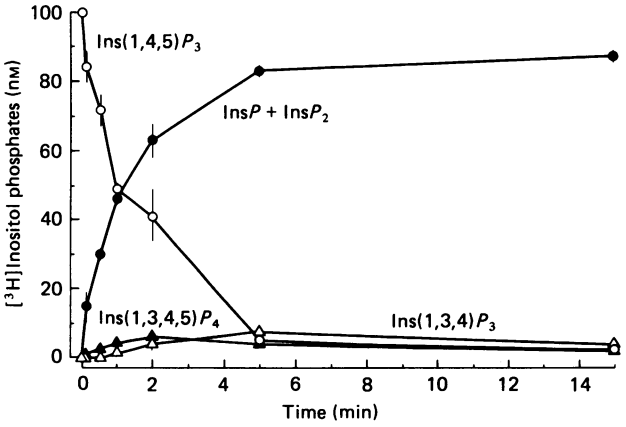

Fig. 3. Time course of metabolism of [^{3}H]Ins(1,4,5)P_3

Permeabilized hepatocytes were incubated with [^{3}H]Ins(1,4,5)P_3 (100 nM) and the inositol phosphates were separated by h.p.l.c. Results show the means ± S.E.M. of three independent experiments with duplicate determinations of each.

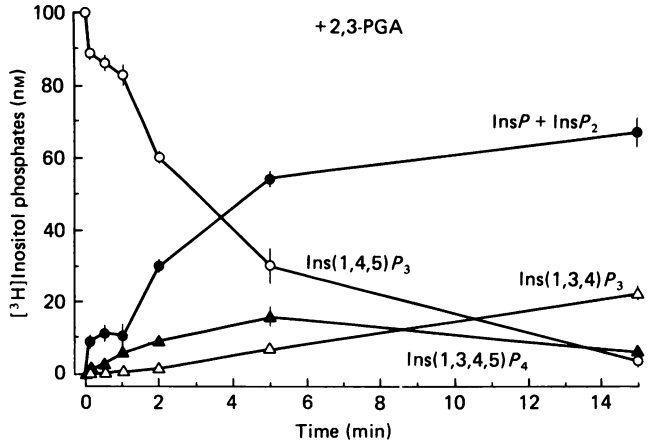

Fig. 4. Effect of 2,3-PGA on the metabolism of [^{3}H]Ins(1,4,5)P_3

Permeabilized hepatocytes were incubated with [^{3}H]Ins(1,4,5)P_3 (100 nM) and 2,3-PGA (10 mM) and the inositol phosphates were separated by h.p.l.c. Results show the means ± S.E.M. of three independent experiments with duplicate determinations for each.

ATP-dependency of Ins(1,4,5)P_3 metabolism

Omission of ATP from the permeabilized hepatocyte preparation completely prevented the formation of [^{3}H]Ins(1,3,4,5)P_4 from [^{3}H]Ins(1,4,5)P_3 (results not shown), indicating, as is expected, the ATP requirement for kinase activity. Also, no radioactivity eluting as Ins(1,3,4)P_3 was formed under these conditions, which is consistent with the idea that the formation of Ins(1,3,4)P_3 occurs as a result of the hydrolysis of Ins(1,3,4,5)P_4 and not by the isomerization of Ins(1,4,5)P_3.

DISCUSSION

As discussed in the Introduction, there is evidence that the Ins(1,3,4)P_3 formed in stimulated cells arises as a result of the sequential phosphorylation (at position 3) and dephosphorylation (at position 5) of Ins(1,4,5)P_3, liberated from PtdIns(4,5)P_2 breakdown (Batty *et al.*,

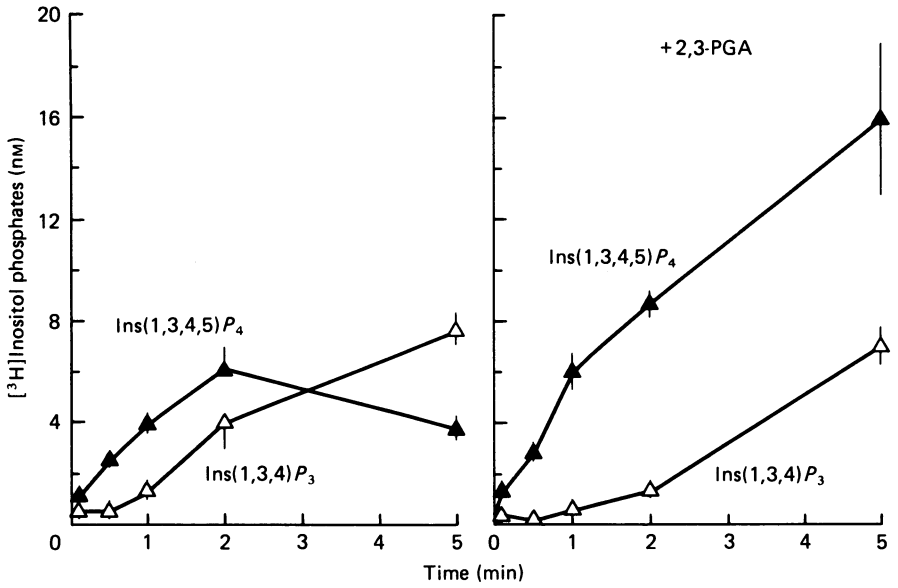

Fig. 5. Comparison of the effect of 2,3-PGA on the formation of [^{3}H]Ins(1,3,4)P_3 and [^{3}H]Ins(1,3,4,5)P_4

Results represent the early time points for the data shown in Fig. 3 and Fig. 4.

1985; Heslop *et al.*, 1985; Irvine *et al.* 1986). The purpose of the experiments reported here was to determine whether such a pathway could be demonstrated in guinea-pig hepatocytes, where significant formation of Ins(1,3,4)P_3 had been previously documented. Saponin-permeabilized cells were used, since this preparation permits access of radiolabelled precursors to the intracellular milieu while at least partially maintaining the structural relationships between cellular compartments (Burgess *et al.*, 1983). The findings may be summarized as follows: (1) in intact [^{3}H]inositol-labelled guinea-pig hepatocytes stimulated with 1 μM-angiotensin II, a ^{3}H-labelled compound was rapidly produced which co-eluted on h.p.l.c. with [^{32}P]Ins(1,3,4,5)P_4; this material is presumed to be Ins(1,3,4,5)P_4, although its precise structure has not been determined; (2) in permeable guinea-pig hepatocytes, [^{3}H]Ins(1,4,5)P_3 was also converted to a [^{3}H]InsP_4 and a ^{3}H-labelled substance which co-elutes on h.p.l.c. with Ins(1,3,4)P_3; (3) [^{32}P]Ins(1,3,4,5)P_4 was apparently converted to a [^{32}P]Ins(1,3,4)P_3 but not to [^{32}P]Ins(1,4,5)P_3; (4) the formation of the presumed [^{3}H]Ins(1,3,4,5)P_4 and [^{3}H]Ins(1,3,4)P_3 from [^{3}H]Ins(1,4,5)P_3 was ATP-dependent; (5) 2,3-PGA partially inhibited the production of [^{3}H]InsP_2 from [^{3}H]Ins(1,4,5)P_3 and the production of [^{32}P]Ins(1,3,4)P_3 from [^{32}P]Ins(1,3,4,5)P_4.

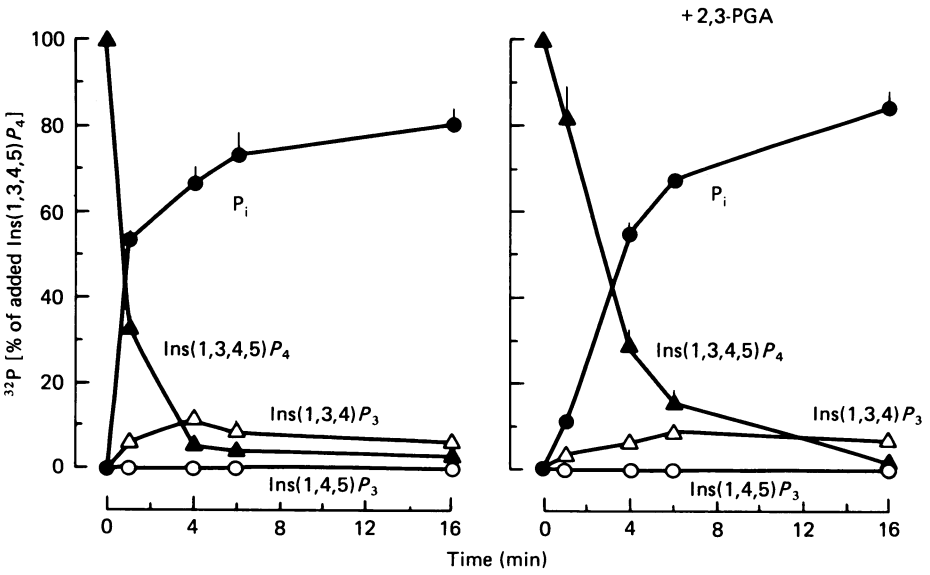

Fig. 6. Metabolism of [^{32}P]Ins(1,3,4,5)P_4

Permeabilized hepatocytes were incubated with 100 nM-Ins(1,4,5)P_3 for 2 min, at which time [^{32}P]Ins(1,3,4,5)P_4 with or without 2,3-PGA (10 mM) was added and the inositol phosphates separated by h.p.l.c. Results are the means ± S.E.M. of three independent experiments with duplicate determinations of each. Each point represents the radioactivity as a percentage of the [^{32}P]Ins(1,3,4,5)P_4 added.

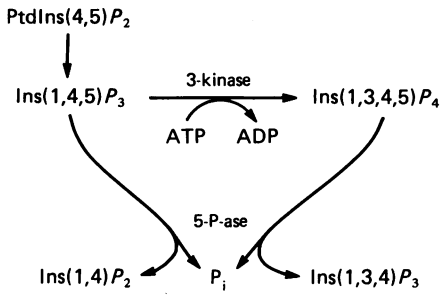

Fig. 7. Metabolism of Ins(1,4,5)P_3 in guinea-pig hepatocytes

Tentatively, it is suggested that the same 5-phosphatase degrades Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4.

Collectively, these results suggest that the pathway to which Ins(1,4,5)P_3 is formed in the guinea-pig hepatocyte is as suggested by Irvine *et al.* (1986). Further, the breakdown of Ins(1,3,4,5)P_4 in these cells appears to be exclusively to the 1,3,4 isomer, indicating that Ins(1,4,5)P_3 is formed directly from the breakdown of PtdIns(4,5)P_2, and not from dephosphorylation of Ins(1,3,4,5)P_4 (Heslop *et al.*, 1985). Since the formation of Ins(1,3,4)P_3 was prevented by omission of ATP, it appears that direct isomerization of Ins(1,4,5)P_3 to Ins(1,3,4)P_3 does not occur, as suggested previously (Burgess *et al.*, 1985).

The initial enzymic reactions involved in inositol polyphosphate metabolism in the guinea-pig hepatocyte as suggested by these data are summarized in Fig. 7. Tentatively, the dephosphorylation of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 is attributed to the same 5-phosphatase which, in the liver, is believed to be a plasma membrane enzyme (Seyfred *et al.*, 1984; Storey *et al.*, 1984). This conclusion is based at present on the observation that these reactions are both partially inhibited by 10 mM-2,3-PGA. However, a more rigorously quantitative assessment of the action of this inhibitor is needed, which was not possible in this study due to the unavailability of chemically pure Ins(1,3,4,5)P_4 for kinetic studies.

The physiological significance of this alternative pathway for Ins(1,4,5)P_3 metabolism in the liver is unknown. The rapid formation of Ins(1,3,4,5)P_4 is consistent with a potential messenger function for this molecule, but rapid phosphorylation of Ins(1,4,5)P_3 could also serve as an efficient mechanism to inactivate Ca^{2+}-releasing activity. Indirect evidence has suggested that Ins(1,3,4)P_3 does not play a direct role in the Ca^{2+}-mobilizing pathway in exocrine pancreas (Burgess *et al.*, 1985). As yet, tests of the biological activity of purified Ins(1,3,4,5)P_4 and Ins(1,3,4)P_3 have not been reported, but results of such experiments will surely be forthcoming in the near future.

After this manuscript was submitted, the results of a similar study using rat hepatocytes appeared, and with similar conclusions (Hansen *et al.*, 1986). Also, Irvine & Moor (1986) have suggested, based on experiments with sea urchin eggs, that Ins(1,3,4,5)P_4 is an intracellular second messenger whose function is to control Ca^{2+} homeostasis at the plasma membrane.

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