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Dephosphorylation of *myo*-inositol 1,4,5-trisphosphate and *myo*-inositol 1,3,4-trisphosphate

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We have augmented our previous studies [Storey, Shears, Kirk & Michell (1984) Nature (London) **312**, 374–376] on the subcellular location and properties of Ins(1,4,5)P_3 (inositol 1,4,5-trisphosphate) phosphatases in rat liver and human erythrocytes. We also investigate Ins(1,3,4)P_3 (inositol 1,3,4-trisphosphate) metabolism by rat liver. Membrane-bound and cytosolic Ins(1,4,5)P_3 phosphatases both attack the 5-phosphate. The membrane-bound enzyme is located on the inner face of the plasma membrane, and there is little or no activity associated with Golgi apparatus. Cytosolic Ins(1,4,5)P_3 5-phosphatase (M_r 77000) was separated by gel filtration from Ins(1,4)P_2 (inositol 1,4-bisphosphate) and inositol 1-phosphate phosphatases (M_r 54000). Ins(1,4,5)P_3 5-phosphatase activity in hepatocytes was unaffected by treatment of the cells with insulin, vasopressin, glucagon or dibutyryl cyclic AMP. Ins(1,4,5)P_3 5-phosphatase activity in cell homogenates was unaffected by changes in [Ca^{2+}] from 0.1 to 2 μM. After centrifugation of a liver homogenate at 100000 *g*, Ins(1,3,4)P_3 phosphatase activity was largely confined to the supernatant. The sum of the activities in the supernatant and the pellet exceeded that in the original homogenate. When these fractions were recombined, Ins(1,3,4)P_3 phosphatase activity was restored to that observed in unfractionated homogenate. Ins(1,3,4)P_3 was produced from Ins(1,3,4,5)P_4 (inositol 1,3,4,5-tetrakisphosphate) and was metabolized to a novel InsP_2 that was the 3,4-isomer. Ins(1,3,4)P_3 phosphatase activity was not changed by 50 mM-Li^+ or 0.07 mM-Ins(1,4)P_2 alone, but when added together these agents inhibited Ins(1,3,4)P_3 metabolism. In Li^+-treated and vasopressin-stimulated hepatocytes, Ins(1,4)P_2 may reach concentrations sufficient to inhibit Ins(1,3,4)P_3 metabolism, with little effect on Ins(1,4,5)P_3 hydrolysis.

INTRODUCTION

Inositol lipid hydrolysis is a widespread mechanism of receptor-coupled transmembrane signalling (reviewed by Michell *et al.*, 1981; Berridge, 1984; Nishizuka, 1984; Downes & Michell, 1985). After cell stimulation, the hydrolysis of PtdIns(4,5)P_2 leads to the rapid accumulation of Ins(1,4,5)P_3, which mobilizes Ca^{2+} from intracellular stores (reviewed by Berridge & Irvine, 1984). Ins(1,4,5)P_3 can be dephosphorylated to Ins(1,4)P_2 by a 5-phosphatase that is present in plasma membranes and cytosol (Downes & Michell, 1981; Seyfred *et al.*, 1984; Storey *et al.*, 1984; Joseph & Williams, 1985; Connolly *et al.*, 1985; Sasaguri *et al.*, 1985; Erneux *et al.*, 1986; Kukita *et al.*, 1986). Since Ins(1,4)P_2 does not mobilize Ca^{2+}, the hydrolysis of the 5-phosphate of Ins(1,4,5)P_3 deactivates the second messenger. It is therefore important to understand the factors which affect the activity of the 5-phosphatase *in vivo*.

We have studied the Ins(1,4,5)P_3 5-phosphatase activity of human erythrocytes and rat liver. The plasma membranes of the former tissue are a convenient source of the enzyme (Downes & Michell, 1981; Downes *et al.*, 1982). In liver, we have been particularly concerned with the possibility that the phosphatase may be regulated after receptor activation. We have also attempted to relate our findings to studies of Ins(1,4,5)P_3 metabolism in intact hepatocytes (Thomas *et al.*, 1984; Burgess *et al.*, 1985; Palmer *et al.*, 1986; Hansen *et al.*, 1986).

The interpretation of these studies has been complicated by the identification of a second pathway of Ins(1,4,5)P_3 metabolism. This involves its phosphorylation to Ins(1,3,4,5)P_4, at least in rat brain and parotid gland (Batty *et al.*, 1985; Irvine *et al.*, 1986*a*; Downes *et al.*, 1986). An InsP_4 which was presumed to be Ins(1,3,4,5)P_4 has also been detected in liver (Hansen *et al.*, 1986; Palmer *et al.*, 1986) and pancreas (Turk *et al.*, 1986). The Ins(1,3,4,5)P_4 is dephosphorylated to Ins(1,3,4)P_3 (Batty *et al.*, 1985; Hansen *et al.*, 1986; Hawkins *et al.*, 1986). This metabolic pathway accounts for the accumulation of Ins(1,3,4)P_3 in a variety of stimulated cells (Irvine *et al.*, 1984, 1985; Batty *et al.*, 1985; Burgess *et al.*, 1985; Hansen *et al.*, 1986; Hawkins *et al.*, 1986; Palmer *et al.*, 1986; Turk *et al.*, 1986). Although it is not known whether Ins(1,3,4)P_3 has any second-messenger activity, it is clearly important in the process by which Ins(1,4,5)P_3 is returned to the free inositol pool. We have therefore studied Ins(1,3,4)P_3 degradation by liver.

Abbreviations used: Ins, Ins$P$, InsP_2, Ins(1,4)P_2, Ins(1,3)P_2, Ins(3,4)P_2, InsP_3, Ins(1,3,4)P_3, Ins(1,4,5)P_3, InsP_4 and Ins(1,3,4,5)P_4 are *myo*-inositol and its mono-, bis-, tris- and tetrakis-phosphate derivatives (locants designated where appropriate, and known or assumed to be D-enantiomers); PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate.

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Some of these data have been reported in preliminary form (Shears *et al.*, 1985; Kirk *et al.*, 1987).

MATERIALS AND METHODS

Preparation and incubation of hepatocytes

Hepatocytes were prepared as described by Shears & Kirk (1984*a*), with the following modifications. The flow rate during the perfusion was maintained at 25 ml/min, and the medium did not contain Tes. The hepatocytes were suspended at approx. 20 mg dry wt./ml in Krebs bicarbonate-buffered media (Krebs & Henseleit, 1932), supplemented with 1.25 mM-$CaCl_2$, 10 mM-glucose and 2% (w/v) bovine serum albumin. The incubations were gassed every 15 min with O_2/CO_2 (19:1).

Studies on the effects of hormones on Ins(1,4,5)P_3 5-phosphatase were performed by first adding the agonists to hepatocyte incubations for an appropriate time. Then EGTA was added to a final concentration of 2 mM, and 0.5 ml samples were immediately frozen and stored in liquid N_2. These samples were subsequently thawed in 2 ml of 0.25 M-sucrose/5 mM-Tris/HCl, pH 8.0, and then homogenized for 10 s with a Polytron homogenizer on setting 5. Samples (100 μl) were then immediately assayed for Ins(1,4,5)P_3 5-phosphatase activity in 1 ml of assay buffer (see below). This procedure diluted the original hepatocyte incubations 55-fold, so the free [Mg^{2+}] and [Ca^{2+}] in the assay medium were not significantly changed by addition of the sample.

Preparation of liver homogenates and soluble and particulate fractions

The liver of a male rat (220 g) was perfused during diethyl ether anaesthesia with ice-cold 0.25 M-sucrose/5 mM-Tes (pH 7.2). Remaining procedures were performed at 0–4 °C. The liver was homogenized (Storey *et al.*, 1984), filtered through gauze and made up to a 30% (w/v) suspension with 0.25 M-sucrose/5 mM-Tes (pH 7.2).

Homogenates were stored on ice until assayed (usually < 1.5 h). Portions of the homogenate (25 ml) were sometimes centrifuged at 100000 ***g*** for 1 h. The resultant supernatant and pellet were both made up to 25 ml with more 0.25 M-sucrose/5 mM-Tes in order to ensure that their dilution, relative to the original tissue, was similar to that of the homogenate.

Preparation of Golgi-rich vesicles

The liver of a male rat (200 g) was perfused during ether anaesthesia with ice-cold 0.25 M-sucrose to remove the blood. Golgi-rich vesicles were prepared by the method of Morré (1971), except that, after the preparation was removed from the interface of the discontinuous sucrose-density gradient, it was washed twice with 0.25 M-sucrose (Kuhn & White, 1977).

Enzyme markers

Galactosyltransferase (EC 2.4.1.22) was assayed as described by Kuhn & White (1977), except that incubations were terminated with EDTA and reaction products were separated on anion-exchange columns (borate form) (Bretz & Staubli, 1977). Alkaline phosphodiesterase 1 (EC 3.1.4.1) was assayed as described by Razzel (1963). Glycogen phosphorylase (EC 2.4.1.1) was assayed as described by Shears & Kirk (1984*b*).

Measurement of InsP_3 hydrolysis

The incubation medium was slightly modified from that previously described (Storey *et al.*, 1984). It contained 70 mM-potassium glutamate, 30 mM-KCl, 10 mM-NaCl, 24 mM-$MgSO_4$ (4 mM free Mg^{2+}), 0.33 mM-$CaCl_2$ (0.1 μM free Ca^{2+}), 1 mM-EGTA, 10 mM-Hepes, pH 7.2, and saponin (0.2 mg/ml). No InsP_4 was formed from InsP_3 in this medium, because ATP was absent (results not shown; and see Irvine *et al.*, 1986*a*). Dithiothreitol, which was reported to stimulate Ins(1,4,5)P_3 5-phosphatase activity (Seyfred *et al.*, 1984), was without effect in our assays (results not shown) and was therefore not included in the incubation medium. For some experiments the free [Ca^{2+}] was altered by changing total [Ca^{2+}]. The media for these studies were prepared at pH 7.2±0.005 and 37 °C by using a Corning 150 pH-meter. The following apparent association constants were used: Ca^{2+}–EGTA, 6.898×10^6, and Mg^{2+}–EGTA, 93.7 (Burgess *et al.*, 1983); Ca^{2+}–glutamate, 26.92, and Mg^{2+}–glutamate, 97.72 (Campbell, 1983). [Ca^{2+}] and [Mg^{2+}] were calculated with the aid of a computer program kindly supplied by Dr. A. Cornish-Bowden (Storer & Cornish-Bowden, 1976).

Approx. 1000 d.p.m. of [4,5-^{32}P]Ins(1,4,5)P_3 (initial concn. 40–300 nM) was added to 0.5 ml incubations. The rate of [4,5-^{32}P]Ins(1,4,5)P_3 hydrolysis was usually assayed by measuring [^{32}P]P_i production (Storey *et al.*, 1984). Alternatively, the disappearance of [4,5-^{32}P]Ins-(1,4,5)P_3 and/or appearance of both [4-^{32}P]Ins(1,4)P_2 and [^{32}P]P_i was measured. In these experiments, incubations were terminated with 0.2 ml of 1.7 M-$HClO_4$. The precipitated protein was removed by centrifugation and the supernatants were neutralized as described by Bone *et al.* (1984), except that 60 mM-EDTA was included in the neutralization medium, to prevent the precipitation of Mg^{2+} salts of InsP_3. After anion-exchange chromatography of the neutralized extracts, [^{32}P]P_i, [4-^{32}P]Ins(1, 4)P_2 and [4,5-^{32}P]Ins(1,4,5)P_3 were sequentially eluted in 20 ml fractions (Downes & Michell, 1981). Radioactivity in these fractions was determined from their Čerenkov radiation.

Hydrolysis of [^{3}H]Ins(1,3,4)P_3 was assayed by measuring the disappearance of substrate. Approx. 1000 d.p.m. of [^{3}H]Ins(1,3,4)P_3 was added to each incubation. We estimate that the initial concentration of Ins(1,3,4)P_3 was 18 nM (see below). The incubations were terminated by $HClO_4$ and neutralized (see above), diluted to 10 ml with water and loaded on columns (2 cm × 0.6 cm) of Bio-Rad AG 1-X8 (200–400 mesh, formate form; Bio-Rad Laboratories, Watford, Herts., U.K.). Then > 99% of Ins$P$ and InsP_2 were eluted with 10 ml of 0.4 M-ammonium formate/0.1 M-formic acid. Finally, > 95% of Ins(1,3,4)P_3 was eluted with 2.5 ml of 2.8 M-ammonium formate/0.1 M-formic acid. This eluate was dissolved in 20 ml of scintillant [2 vol. of xylene, containing 0.8% (w/v) 2,5-diphenyloxazole and 0.02% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)benzene, mixed with 1 vol. of Triton X-100]. The resulting solutions were stable provided that their temperature was kept between 4° and 30 °C. Radioactivity was measured by liquid-scintillation spectrometry.

Preparation of inositol phosphates

[4,5-^{32}P]Ins(1,4,5)P_3 was prepared from [^{32}P]P_i-labelled erythrocytes (Downes & Michell, 1981). [^{3}H]Ins(1,3,4)P_3 was prepared in 2 ml suspensions of rat hepatocytes (see

above) containing 125 μCi of *myo*-[2-^{3}H]inositol/ml. After 90 min, 20 mM-LiCl was added, followed 10 min later by 230 nM-vasopressin. After a further 10 min the incubation was terminated with $HClO_4$, and an InsP_3 fraction, containing Ins(1,4,5)P_3 and Ins(1,3,4)P_3, was prepared by anion-exchange chromatography (Palmer *et al.*, 1986). This fraction was freeze-dried (Downes & Michell, 1981). The Ins(1,4,5)P_3 was then removed by incubating the extract with human erythrocyte membranes (5 mg of protein/ml; Downes & Michell, 1981) for 2 h in 2 ml of 20 mM-Hepes (pH 7.0)/5 mM-$MgCl_2$ containing 0.2 mg of saponin/ml. The reaction was terminated with 0.8 ml of 2 M-$HClO_4$, and an InsP_3 fraction (containing approx. 200000 d.p.m. of ^{3}H) was eluted and freeze-dried as described above. The final preparation was taken up in 1 ml of water and stored in batches at −20 °C. The InsP_3 was analysed by h.p.l.c. (see below) and eluted as a single peak with the expected mobility of Ins(1,3,4)P_3 (see, e.g., Fig. 4).

The chemical quantity of Ins(1,3,4)P_3 produced by this method was estimated to be about 1.8 nmol, assuming that vasopressin-stimulated and Li^+-treated hepatocytes produce 60 pmol of [^{3}H]InsP_3/mg cell dry wt. (Thomas *et al.*, 1984), of which 75% was assumed to be Ins(1,3,4)P_3 (Hansen *et al.*, 1986). Since 5 μl of the final preparation (in 1 ml) was usually added to 0.5 ml incubations for the assay of Ins(1,3,4)P_3 phosphatase activity, the initial Ins(1,3,4)P_3 concentration therein would be about 18 nM.

[^{3}H]InsP_4 and [4,5-^{32}P]InsP_4 were respectively prepared by ATP-dependent phosphorylation (Irvine *et al.*, 1986*a*) of either 1 μCi of [^{3}H]Ins(1,4,5)P_3 (nominally 1 Ci/mmol; Amersham International) or 0.1 μCi of [4,5-^{32}P]Ins-(1,4,5)P_3 (prepared from human erythrocytes; Downes *et al.*, 1982). The InsP_3 was incubated at 37 °C with 2% (v/v) of a 100000 *g* rat liver supernatant in 0.5 ml of medium containing 0.25 M-sucrose, 50 mM-Tes (pH 7.5) 20 mM-$MgCl_2$, 10 mM-ATP, 25 mM-2,3-bisphosphoglycerate (Na^+ salt) and saponin (0.2 mg/ml). After 15 min (for incubations containing ^{32}P) or 45 min (for incubations with ^{3}H), reactions were terminated with 0.2 ml of 1.7 M-$HClO_4$. After neutralization (see above), the InsP_4 was purified by anion-exchange chromatography (Batty *et al.*, 1985) and freeze-dried (Downes & Michell, 1981). Samples were taken up in 1 ml of water and stored in batches at −20 °C. In both preparations, about 50% of the starting InsP_3 was recovered as InsP_4. Some batches of [^{3}H]InsP_3 yielded less InsP_4, apparently because of batch variation in specific radioactivity, which affected the InsP_3 concentration in our medium (nominally 2 μM, and close to the K_m for the kinase of 0.6 μM; see Irvine *et al.*, 1986). Percentage yields were restored when only 0.5 μCi of [^{3}H]InsP_3 was added. We have assumed that our preparations of InsP_4 are the 1,3,4,5 isomer, as in brain and parotid glands (Batty *et al.*, 1985; Downes *et al.*, 1986; Irvine *et al.*, 1986*a*). Consistent with this assumption, the InsP_4 was eluted as a single peak on h.p.l.c. with the expected mobility of Ins(1,3,4,5)P_4 (results not shown).

[4-^{32}P]Ins(1,4)P_2 was prepared by 5-phosphatase hydrolysis of [4,5-^{32}P]Ins(1,4,5)P_3 and freeze-dried (Downes & Michell, 1981). Non-radioactive Ins(1,4)P_2 was generously supplied by Dr. R. Irvine.

Preparation of soluble Ins(1,4,5)P_3 phosphatase

The liver of a male rat (250 g) was perfused with ice-cold 0.25 M-sucrose/5 mM-Tris/HCl, pH 7.4, under pentobarbitone anaesthesia. Once cleared of blood, the liver was homogenized in an equal volume of 0.25 M-sucrose/5 mM-Tris/HCl, pH 7.4, filtered and centrifuged at 0–4 °C for 1 h at 180000 *g*. The supernatant was concentrated about 3-fold by using Amicon Centricon-10 micro-concentrators, and 1 ml of the resultant preparation was applied to a column (75 cm × 4 cm) of Sephadex G-100 equilibrated with 20 mM-maleate/5 mM-Tris/HCl, pH 7.2, and eluted with the same medium at a flow rate of 0.2 ml/min. The column was calibrated with carbonic anhydrase, creatine kinase and bovine serum albumin (M_r 81000, 66000 and 29000 respectively).

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

Incubations to be analysed by h.p.l.c. were terminated with 0.2 ml of ice-cold 1.7 M-$HClO_4$. Samples were then prepared, at 0–4 °C, by a slight modification of a method (Downes *et al.*, 1986) in turn derived from that of Sharpes & McCarl (1982). The tissue pellets were removed by centrifugation for 5 min at 1000 *g*. Then 0.65 ml of the supernatant was added to 1 ml of 1,1,2-trichlorotrifluoroethane/tri-n-octylamine (1:1, v/v). After vigorous mixing, samples were centrifuged for 5 min at 1000 *g*. Next, 0.4 ml of the upper aqueous phase was removed, and replaced with 0.4 ml of water. The mixing and centrifugation were repeated, and 0.4 ml of the aqueous layer was removed. The combined aqueous samples were filtered (0.45 μm filters, Gelman Sciences Ltd., Northampton, U.K.) and loaded, via a 1 ml injection loop, on to a 25 cm × 0.46 cm main column plus 5 cm × 0.46 cm guard column, both containing Whatman Partisil 10-SAX. Elution began with water, for 5 min, and then exactly followed the protocol of Batty *et al.* (1985), at a flow rate of 1.25 ml/min. The 'dead' time in our apparatus was 2.5 min (i.e. the time between injection of [^{3}H]inositol and its appearance in the fractions; Fig. 4). Fractions (1 ml) were collected for the first 10 min, and 0.5 ml of each of these was added to 5 ml of scintillant (see above). For the remainder of the elution period 0.25 ml fractions were collected. These were dissolved in 1 ml of water/methanol (1:1, v/v) (see Hawkins *et al.*, 1986), followed by 4 ml of scintillant (see above). Radioactivity was measured by liquid-scintillation spectrometry.

Materials

Radiochemicals were purchased from Amersham International (Amersham, Bucks., U.K.). Saponin was supplied by Cambridge Bioscience (Cambridge, U.K.). ATP, 2,3-bisphosphoglycerate (Na^+ salt), Hepes, hormones and Tes were from Sigma Chemical Co. (Poole, Dorset, U.K.). EGTA was from Fluka (Buchs, Switzerland). The $CaCl_2$ used for the Ca^{2+}/EGTA buffers was made by adding HCl to $CaCO_3$. 1,1,2-Trichlorotrifluoroethane and tri-n-octylamine were purchased from Aldrich Chemical Co. (Gillingham, Dorset, U.K.).

RESULTS AND DISCUSSION

Properties of the Ins(1,4,5)P_3 5-phosphatase of the plasma membrane

In our previous studies of the Ins(1,4,5)P_3 5-phosphatase from erythrocytes (Downes *et al.*, 1982), assays were performed in a dilute buffer (30 mM-Hepes/2 mM-$MgCl_2$). In recognition of the probable regulatory importance of this enzyme, we changed to the

routine use of a medium that more closely resembles cell cytosol (see the Materials and methods section). When erythrocyte membranes were incubated in the medium of nearer-physiological ionic strength, the first-order rate constant for Ins(1,4,5)P_3 hydrolysis was $60 \pm 4\%$ ($n = 4$) of that observed in the more dilute medium. Therefore, those workers who have used low-ionic-strength media (Seyfred *et al.*, 1984; Connolly *et al.*, 1985; Erneux *et al.*, 1986) may have overestimated the physiological activity of the 5-phosphatase.

Our preparations of [4,5-^{32}P]Ins(1,4,5)P_3 (see the Materials and methods section) contained a greater proportion of ^{32}P in the 5-phosphate than in the 4-phosphate. When the 5-phosphate alone was removed from a batch of [4,5-^{32}P]Ins(1,4,5)P_3 by incubating it with erythrocyte membranes (Downes *et al.*, 1982), the ratio for the distribution of ^{32}P label in the products, [^{32}P]P_i: [4-^{32}P]Ins(1,4)P_2, was 2.4:1 ($n = 2$). Liver plasma membranes (prepared as described by Storey *et al.*, 1984) were incubated with the same batch of unevenly labelled [4,5-^{32}P]Ins(1,4,5)P_3. During 15 min incubations there was no significant hydrolysis of the InsP_2 produced, and the ^{32}P label was distributed between the products [^{32}P]P_i and [^{32}P]InsP_2 in the ratio 2.5:1 ($n = 2$). Therefore the InsP_2 can only be the 1,4-isomer, confirming that the Ins(1,4,5)P_3 phosphatase of liver plasma membranes specifically attacks the 5-phosphate. Two other reports (Seyfred *et al.*, 1984; Joseph & Williams, 1985) noted that the hydrolysis of [4,5-^{32}P]Ins(1,4,5)P_3, by liver plasma membranes, yielded more ^{32}P label in P_i than in Ins(1,4)P_2. However, these experiments did not give a quantitative assessment of phosphates removed from [4,5-^{32}P]Ins(1,4,5)P_3, because the relative radioactivities of the 4- and 5-phosphates were not measured.

In a previous study (Storey *et al.*, 1984) we showed that approximately three-quarters of the hepatic Ins(1,4,5)P_3 5-phosphatase activity was membrane-bound, and the remainder was cytosolic. We also separated liver fractions on Percoll density gradients, and Ins(1,4,5)P_3 5-phosphatase was associated with markers for plasma membranes, but not with markers for lysosomes, endoplasmic reticulum or mitochondria (Storey *et al.*, 1984). However, in these experiments we did not separate plasma membranes from Golgi membranes. Two other groups of workers (Seyfred *et al.*, 1984; Joseph & Williams, 1985) also showed that Ins(1,4,5)P_3 5-phosphatase co-purified with markers for plasma membranes, but not for endoplasmic reticulum or mitochondria. Hence it has not previously been shown to what extent the Golgi apparatus can dephosphorylate Ins(1,4,5)P_3. We have now resolved this uncertainty by purifying a Golgi-rich fraction from rat liver. This fraction showed an 86-fold purification of a Golgi marker (galactosyltransferase) relative to the homogenate (Table 1). However, the enrichment of Ins(1,4,5)P_3 5-phosphatase in the Golgi fractions was only 4.3-fold, which is of the same order as the 2.7-fold enrichment of the plasma-membrane marker alkaline phosphodiesterase 1. The data in Table 1 demonstrate that there was little Ins(1,4,5)P_3 5-phosphatase activity in Golgi vesicles.

We have measured Ins(1,4,5)P_3 5-phosphatase activity on the inner and outer faces of tightly resealed erythrocyte membranes. The efficiency of resealing in these experiments was confirmed by the almost complete latency of the Ca^{2+}/calmodulin-activated Ca^{2+}-ATPase, an enzyme with its active site on the cytoplasmic face of the membrane (results not shown; and see Downes *et al.*, 1981). The Ins(1,4,5)P_3 5-phosphatase activity of these resealed membranes was very low, and was activated 70-fold by small quantities of saponin (Fig. 1). It thus appears that virtually all of the Ins(1,4,5)P_3 5-phosphatase activity in erythrocyte membranes is at the cytoplasmic surface. Since Ins(1,4,5)P_3 is largely retained

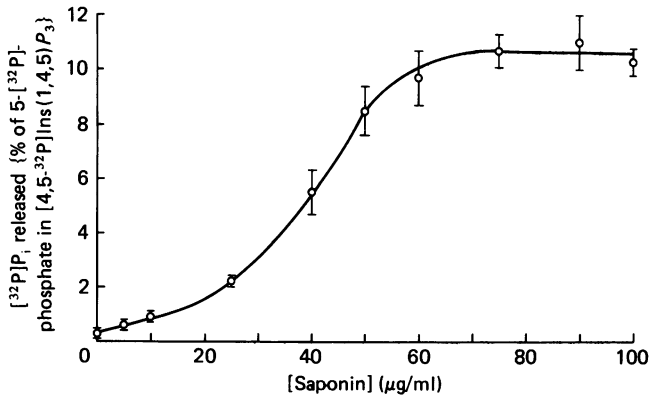

Fig. 1. Effect of saponin on Ins(1,4,5)P_3 5-phosphatase in resealed erythrocyte ghosts

Resealed erythrocyte ghosts (Billah *et al.*, 1977) were incubated for 5 min in a volume of 1 ml at 37 °C with 3000–4000 d.p.m. of [4,5-^{32}P]Ins(1,4,5)P_3. Hydrolysis of Ins(1,4,5)P_3 was assayed by measuring [^{32}P]P_i release (see the Materials and methods section). Data are means ±S.E.M. for three to eight experiments.

Table 1. Ins(1,4,5)P_3 5-phosphatase in rat liver Golgi fractions

The Ins(1,4,5)P_3 5-phosphatase activity was assayed by measuring [^{32}P]P_i release. The specific radioactivity of Ins(1,4,5)P_3 was calculated as previously described (Downes & Michell, 1981). Less than 0.05 mg of protein/ml was added to the incubations, under conditions in which the initial reaction rate was proportional to protein concentration. Other conditions are as described in the Materials and methods section. Data represent means of two experiments.

	Activity (nmol/min per mg of protein)		
	Ins(1,4,5)P_3 5-phosphatase	Galactosyltransferase	Alkaline phosphodiesterase 1
Homogenate	2.81	4.8	130
Golgi membranes	12.0	412	350
Enrichment factor	4.3	85.8	2.7

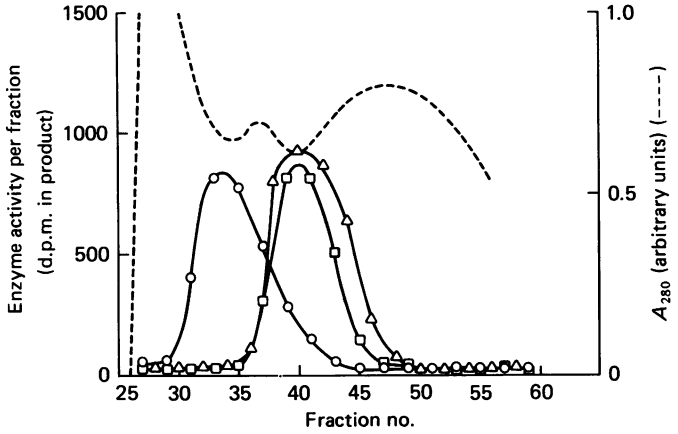

Fig. 2. Gel filtration of soluble inositol phosphate phosphatases

A rat liver soluble preparation was fractionated on a Sephadex G-100 column as described in the Materials and methods section. Samples from 5 ml fractions were incubated at 37 °C with various substrates. Data represent [^{32}P]P_i release (Storey *et al.*, 1984) from either [4,5-^{32}P]Ins(1,4,5)P_3 (○) or [4-^{32}P]Ins(1,4)P_2 (△), or [^{14}C]Ins release from [^{14}C]Ins1P (□). Results are from a single experiment representative of three.

within hepatocytes (Palmer *et al.*, 1986), it would be expected that, in these cells, Ins(1,4,5)P_3 5-phosphatase would also be located on the inner face of plasma membranes.

The cytosolic Ins(1,4,5)P_3 phosphatase

Cytosolic Ins(1,4,5)P_3 phosphatase was separated from Ins(1,4)P_2 4-phosphatase and Ins1P phosphatase by using gel filtration (Fig. 2). The apparent M_r values of the last two enzymes were about 54000, whereas that of the Ins(1,4,5)P_3 phosphatase was 77000. In contrast, Connolly *et al.* (1985) reported that the cytosolic Ins(1,4,5)P_3 5-phosphatase from platelets had an M_r of 38000. This apparent difference may indicate that this enzyme can exist in a multimeric form. Alternatively, the Ins(1,4,5)P_3 5-phosphatases of these two cell types may be quite distinct.

A preparation of soluble Ins(1,4,5)P_3 phosphatase freed of Ins(1,4)P_2 phosphatase (as in Fig. 2) was incubated with asymmetrically labelled [4,5-^{32}P]Ins(1,4,5)P_3. The ratio of ^{32}P label in the products, [^{32}P]P_i : [^{32}P]InsP_2, was 2.3 ± 0.2:1 (n = 3). This batch of [4,5-^{32}P]Ins(1,4,5)P_3 (since it was different from that used in the study of the 5-phosphatase of liver plasma membranes described above) was also incubated with erythrocyte membranes to remove the 5-phosphate (Downes *et al.*, 1982). ^{32}P label was distributed between the products with a [^{32}P]P_i: [4-^{32}P]Ins(1,4)P_2 ratio of 2.1 ± 0.2:1 (n = 3). Thus the cytosolic Ins(1,4,5)P_3 phosphatase selectively hydrolyses the 5-phosphate.

Is Ins(1,4,5)P_3 5-phosphatase activity regulated by hormones?

Suspensions of hepatocytes were challenged for 0.5–5 min with various agonists, each chosen because of its different mode of signal transduction. Samples of these cells were then analysed for Ins(1,4,5)P_3 5-phosphatase activity (see the Materials and methods section); 10 nM-insulin, 230 nM-vasopressin, 10 nM-glucagon or 100 μM-dibutyryl cyclic AMP did not alter the rate of Ins(1,4,5)P_3 hydrolysis (Table 2), even if, in both the assay medium and the medium used to homogenize the hepatocytes, 1 mM-F^- was included. Higher [F^-] was not added, since this would have directly inhibited Ins(1,4,5)P_3 5-phosphatase (Storey *et al.*, 1984). Further assays, on samples taken from incubations of hepatocytes treated with 230 nM-vasopressin for 2 min, demonstrated that glycogen phosphorylase activity was 3.7 ± 0.5-fold (n = 4) greater than in control samples. This stimulation is of a similar magnitude to that observed in our previous experiments (Shears & Kirk, 1984*b*), indicating that these cells responded normally to vasopressin.

Table 2. Studies on potential short-term modulators of Ins(1,4,5)P_3 5-phosphatase activity in hepatocytes

Hepatocyte incubations, treatment with hormones (for 2 min) and assays of [^{32}P]P_i release from [4,5-^{32}P]-Ins(1,4,5)P_3 were carried out as described in the Materials and methods section. Data are means ± S.E.M. (where appropriate) from two to four experiments: n.d., not determined. Similar results were obtained when hepatocytes were treated with the hormones for 0.5 and 5 min.

	Rate constant of Ins(1,4,5)P_3 5-phosphatase (% of control) at various $[Ca^{2+}]_{free}$			
	1 nM	0.1 μM	0.6 μM	2 μM
Control	100 ± 3	93 ± 4	86 ± 8	105 ± 4
Insulin (10 nM)	94 ± 4	107 ± 6	82 ± 13	n.d.
Vasopressin (230 nM)	108 ± 5	100	100	n.d.
Glucagon (10 nM)	93 ± 7	103 ± 8	99 ± 9	n.d.
Dibutyryl cyclic AMP (100 μM)	94	n.d.	n.d.	99

The cytosolic [Ca^{2+}] in unstimulated rat hepatocytes is about 0.16 μM and increased to about 1.3 μM during maximal stimulation with agonists (Berthon *et al.*, 1984; Thomas *et al.*, 1984). Table 2 shows that increasing the [Ca^{2+}] in our assay medium from 0.1 μM up to 2 μM did not affect Ins(1,4,5)P_3 5-phosphatase activity (even if 10 μg of calmodulin/ml was also added; results not shown). These experiments suggest that the Ins(1,4,5)P_3 5-phosphatase of liver is not regulated by hormonal mobilization of Ca^{2+}. In contrast, Ins(1,4,5)P_3 5-phosphatase activity in pig smooth muscle (Sasaguri *et al.*, 1985) and guinea-pig macrophages (Kukita *et al.*, 1986) was stimulated substantially when the [Ca^{2+}] was increased from 0.1 to 1 μM. There may therefore be tissue- and species-dependent differences in the Ca^{2+}-sensitivity of Ins(1,4,5)P_3 hydrolysis. In another study, Connolly *et al.* (1985) demonstrated that Ca^{2+}, with a K_i of 70 μM, inhibited the activity of Ins(1,4,5)P_3 5-phosphatase prepared from platelets. However, this Ca^{2+} concentration is too high to be of physiological significance.

Treatment of cell homogenates with 100 μM-5′-guanylyl imidodiphosphate did not affect Ins(1,4,5)P_3 5-phosphatase activity (results not shown), suggesting that this enzyme is not regulated by GTP-binding proteins of the type implicated in the control of adenylate cyclase activity.

Ins(1,3,4)P_3 metabolism

Diluted homogenates of liver (0.17%, v/v) were incubated in the medium of near-physiological ionic strength, containing 0.1 μM-Ca^{2+} and trace quantities of [^{3}H]Ins(1,3,4)P_3. In these incubations 50% of the substrate was hydrolysed in 7–8 min (Fig. 3*a*), although not with first-order kinetics (results not shown). This rate of hydrolysis was half that observed for Ins(1,4,5)P_3 hydrolysis by similar dilutions of liver homogenate (Storey *et al.*, 1984). Ins(1,3,4)P_3 metabolism was also studied in incubations containing 100000 *g* soluble and particulate fractions of liver. These fractions were adjusted to their concentration in the original homogenates (see the Materials and methods section). Ins(1,3,4)P_3 phosphatase activity predominated in the supernatant fraction, and there was much less activity in the particulate fraction (Fig. 3*a*). Although in some experiments the soluble Ins(1,3,4)P_3 phosphatase activity exceeded that of the original homogenate, overall this was not a statistically significant phenomenon. Nevertheless, the total activity of the particulate and soluble fractions always exceeded that of the original homogenate (Fig. 3*a*). Therefore, the Ins(1,3,4)P_3 phosphatase activity must be activated in some way during the separation of the particulate and soluble fractions. When these fractions were recombined, this apparent activation was reversed, and the total Ins(1,3,4)P_3 phosphatase activity was restored to that in the unfractionated homogenate (Fig. 3*b*). We do not know whether this reversible activation of Ins(1,3,4)P_3 phosphatase represents a mechanism of physiological significance.

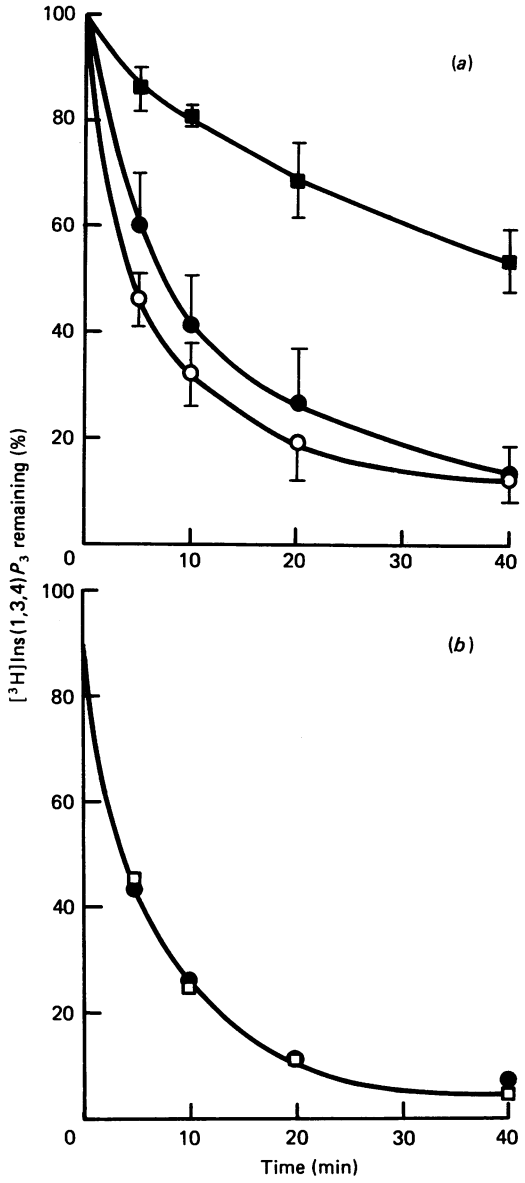

Fig. 3. Rates of Ins(1,3,4)P_3 metabolism by liver homogenate and by 100000 *g* soluble and particulate fractions

Samples (25 μl) of liver homogenate (●) and of 100000 *g* soluble (○) and particulate (■) fractions were incubated with [^{3}H]Ins(1,3,4)P_3 for various times as described in the Materials and methods section. Results are means ± S.E.M. for five experiments (*a*). The sum of the soluble and particulate activities always exceeded that of the original homogenate. In two of these experiments (*b*), the soluble and particulate fractions were recombined in the proportions present in the original homogenate. Samples (25 μl) of the recombined fractions (□) and the original unfractionated homogenate (●) were then immediately incubated.

Products of Ins(1,3,4)P_3 metabolism

The ^{3}H-labelled products of [^{3}H]Ins(1,3,4)P_3 metabolism were separated, by h.p.l.c., into three fractions (Fig. 4). The first two fractions had the expected mobilities of Ins and Ins*P* respectively. In samples taken from incubations terminated at 10 min (results not shown) and 20 min (Fig. 4*b*), no ^{3}H-labelled product co-chromatographed with authentic Ins(1,4)P_2. However, a ^{3}H-labelled compound, which was presumably another isomer of InsP_2, was eluted 0.6 min after Ins(1,4)P_2.

In principle, the novel InsP_2 could be Ins(3,4)P_2 or Ins(1,3)P_2, or a mixture of both. Other studies, with liver (Hansen *et al.*, 1986) and parotid glands (Hawkins *et al.* 1986), have also demonstrated that the major ^{3}H-labelled product of Ins(1,3,4)P_3 hydrolysis is not Ins(1,4)P_2. Hansen *et al.* (1986) suggested that the novel isomer was Ins(1,3)P_2, but reported no results to justify this proposal.

We further analysed the novel InsP_2 isomer derived from Ins(1,3,4)P_3 by studying the simultaneous dephosphorylation of [4-^{32}P]Ins(1,3,4)P_3 and [^{3}H]Ins(1,3,4)P_3. These substrates were prepared by incubating [4,5-^{32}P]Ins(1,3,4,5)P_4 and [^{3}H]Ins(1,3,4,5)P_4 with liver homogenate. Other workers have demonstrated that Ins(1,3,4,5)P_4 is converted exclusively into Ins(1,3,4)P_3 (Batty *et al.*, 1985; Hawkins *et al.*, 1986; Hansen *et al.* 1986). This finding was checked in our experiments after first determining that the ^{32}P label in the [4,5-^{32}P]Ins(1,3,4,5)P_4 was distributed between the 5- and 4-phosphates in the ratio 71 ± 0.5 : 29 ± 0.6 (n = 4). This ratio was obtained from the distribution of ^{32}P label between the [^{32}P]P_i and [4-^{32}P]Ins(1,4)P_2 that were produced on incubation of erythrocyte membranes with the [4,5-^{32}P]Ins(1,4,5)P_3 (Downes *et al.*, 1982), from which the [4,5-^{32}P]Ins(1,3,4,5)P_4 was prepared (see the Materials and methods section). If Ins(1,3,4)P_3 was the only InsP_3 isomer to accumulate after dephosphorylation of a mixture of [4,5-^{32}P]Ins(1,3,4,5)P_4 and [^{3}H]Ins(1,3,4,5)P_4, then the ^{32}P:^{3}H ratio of the InsP_3 product would be 29% that of the InsP_4 precursor. This prediction was confirmed by the data in Table 3, where the ^{32}P/^{3}H ratio

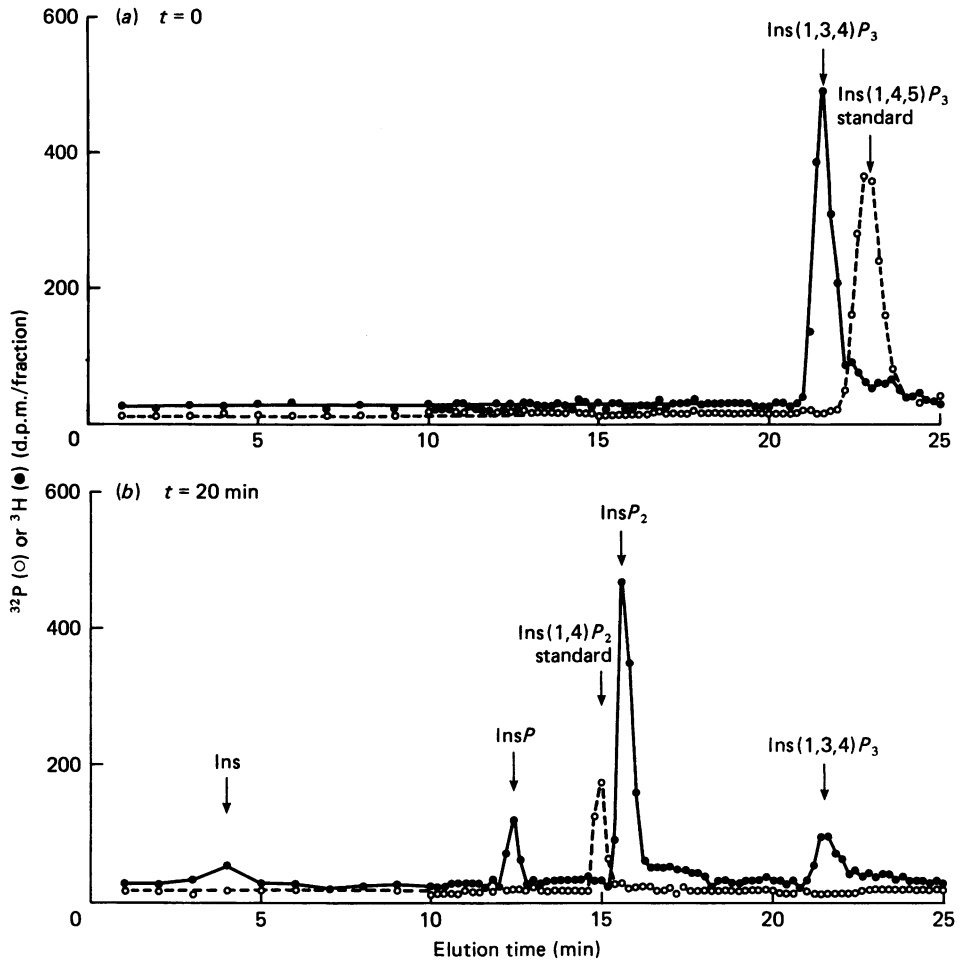

Fig. 4. Products of Ins(1,3,4)P_3 metabolism

Samples (25 μl) of liver homogenate were incubated with [^{3}H]Ins(1,3,4)P_3 for 0 (*a*) and 20 min (*b*) and samples were prepared for h.p.l.c. as described in the Materials and methods section. Immediately before h.p.l.c., the zero-time sample was 'spiked' with [4,5-^{32}P]Ins(1,4,5)P_3 and the 20 min sample was 'spiked' with [4-^{32}P]Ins(1,4)P_2. ●, ^{3}H; ○, ^{32}P (d.p.m./fraction). Note that the peaks 'tailed' slightly before radioactivity returned to the baseline, owing to the age of the column. Two other experiments gave identical results.

of the InsP_3 product was 28–31% of that for Ins(1,3,4,5)P_4. Note that any significantly larger value for the ^{32}P/^{3}H ratio of the InsP_3 fraction would have demonstrated that at least one of the products of Ins(1,3,4,5)P_4 degradation retained the highly labelled 5-phosphate.

After 10 min incubations with liver homogenate, the InsP_2 product retained the same ^{32}P:^{3}H ratio as the Ins(1,3,4)P_3 (Table 3), so the InsP_2 must quantitatively retain the ^{32}P-labelled 4-phosphate. Since Ins(1,4)P_2 is not produced by Ins(1,3,4)P_3 hydrolysis (Fig. 4), the product that accumulates must be Ins(3,4)P_2. The slight decrease in the ^{32}P:^{3}H ratio of the InsP_2 fraction after 20 min incubations (Table 3), which might suggest a small accumulation of Ins(1,3)P_2, was not statistically significant.

The Ins(3,4)P_2 can be separated from Ins(1,4)P_2 by h.p.l.c. (Fig. 4*b*). Therefore, if ^{3}H-labelled inositol phosphates in stimulated cells were to be analysed by h.p.l.c., more than one InsP_2 peak might be detected. This pattern has been observed in experiments on a pituitary cell line (Heslop *et al.*, 1985), pancreas (Turk *et al.*, 1986) and liver (Hansen *et al.*, 1986). The last two groups of workers speculated that Ins(1,3)P_2 was the later-eluted and rather smaller of the two InsP_2 peaks. Our experiments indicate that, at least in liver, the two isomers probably correspond to Ins(1,4)P_2, produced from Ins(1,4,5)P_3, and Ins(3,4)P_2, derived from Ins(1,3,4)P_3.

The ^{32}P:^{3}H ratio of the 'Ins$P$+P$_i$' fraction was much larger than that of the other fractions in Table 3, because the former contained not only [^{32}P]Ins$P$, but also [^{32}P]P$_i$ derived largely from the highly labelled 5-phosphate of [4,5-^{32}P]Ins(1,3,4,5)P_4.

Effects of Li^+ and Ins(1,4)P_2 on InsP_3 phosphatases

In stimulated cells, the concentrations of Ins$P$ and InsP_2 are increased by treatment with Li^+ (Berridge *et al.*, 1982; Thomas *et al.*, 1984; Palmer *et al.*, 1986), which inhibits Ins1P and Ins(1,4)P_2 phosphatases (Hallcher & Sherman, 1980; Berridge *et al.*, 1982; Storey *et al.*, 1984). Li^+ neither potentiates the agonist-mediated accumulation of Ins(1,4,5)P_3 (Burgess *et al.*, 1985; Hansen *et al.*, 1986) nor inhibits Ins(1,4,5)P_3 5-phosphatase (Seyfred *et al.*, 1984; Storey *et al.*, 1984; Joseph & Williams, 1985; Table 4). However, Ins(1,3,4)P_3 concentrations in stimulated cells are increased by Li^+ (Burgess *et al.*, 1985; Hansen *et al.*, 1986), and the ion has also been claimed

Table 3. Metabolism of [^{3}H]Ins(1,3,4,5)P_4 and [4,5-^{32}P]Ins(1,3,4,5)P_4 by liver homogenate

Incubations (0.5 ml) were similar to those described for the measurement of InsP_3 hydrolysis (see the Materials and methods section), except they initially contained 5 μl of the 30% (w/v) liver homogenate, 10 nCi of [^{3}H]Ins(1,3,4,5)P_4 and 3 nCi of [^{32}P]Ins(1,3,4,5)P_4. At the indicated times, samples were acid-quenched, neutralized and loaded on to columns (2 cm × 0.6 cm) of Bio-Rad AG 1-X8 (200–400 mesh; formate form) as described in the Materials and methods section. The following fractions were sequentially eluted: Ins$P$ (0.18 M-ammonium formate), InsP_2 (0.4 M-ammonium formate/0.1 M-formic acid), InsP_3 (0.8 M-ammonium formate/0.1 M-formic acid), InsP_4 (1.2 M-ammonium formate/0.1 M-formic acid). Each eluate consisted of four 2.5 ml fractions which were all dissolved in scintillant and their radioactivities determined (see the Materials and methods section). Appropriate standards indicated that the recovery in each fraction exceeded 97%. Data represent means ± S.E.M. for four experiments. Values in parentheses are percentages of the ^{32}P : ^{3}H ratios of the InsP_4 fractions.

		Recovery of ^{3}H label (% of that in [^{3}H]Ins(1,3,4,5)P_4 added)			^{32}P:^{3}H		
Fraction	Time . . .	0 min	10 min	20 min	0 min	10 min	20 min
Ins$P$ + P_i		0	1±0.5	2±1	–	24±11	14±3
InsP_2		0	11±2	25±5	–	0.076±0.007 (32%)	0.056±0.006 (23%)
InsP_3		2±0.2	48±4	55±3	–	0.074±0.006 (31%)	0.066±0.005 (28%)
InsP_4		98±0.2	38±6	19±4	0.24±0.03 (100%)	0.24±0.03 (100%)	0.24±0.02 (100%)

to inhibit Ins(1,3,4)P_3 hydrolysis (Hansen *et al.*, 1986). In contrast, 50 mM-Li$^+$ did not inhibit Ins(1,3,4)P_3 metabolism in our experiments (Table 4). We do not understand why our result is different from that of Hansen *et al.* (1986).

The Ins(1,4,5)P_3 5-phosphatase prepared from platelets was inhibited by 0.1 mM-Ins(1,4)P_2 (Connolly *et al.*, 1985), but 0.4 mM-Ins(1,4)P_2 did not inhibit Ins(1,4,5)P_3 hydrolysis by erythrocyte membranes (Downes *et al.*, 1982). We studied the effect of 0.07 mM-Ins(1,4)P_2 on hydrolysis of both Ins(1,4,5)P_3 and Ins(1,3,4)P_3, in both the presence and the absence of 50 mM-Li$^+$. This concentration of Ins(1,4)P_2 is within the range of concentrations that we have estimated to occur in Li$^+$-treated and maximally stimulated liver cells. Under such conditions, [Ins(1,4)P_2] may range between 0.03 and 0.2 mM, assuming that [InsP_2] is predominantly that of the 1,4-isomer and is 2–4 × [InsP_3], which may be 0.016–0.05 mM (Burgess *et al.*, 1984; Thomas *et al.*, 1984; Hansen *et al.*, 1986; Palmer *et al.*, 1986). Although 0.07 mM-Ins(1,4)P_2 alone may have slightly inhibited the dephosphorylation of Ins(1,4,5)P_3 and Ins(1,3,4)P_3, these effects did not achieve statistical significance (Table 4). However, in these incubations the concentration of Ins(1,4)P_2 would have decreased rapidly, owing to its dephosphorylation (Storey *et al.*, 1984). When Ins(1,4)P_2 hydrolysis was greatly decreased by including 50 mM-Li$^+$ in the incubations (see Storey *et al.*, 1984), the metabolism of both InsP_3 isomers was inhibited significantly (Table 4). The effect on Ins(1,3,4)P_3 hydrolysis was considerably greater than that on Ins(1,4,5)P_3 metabolism, although a precise quantitative comparison is not possible because of the unspecified kinetic properties of Ins(1,3,4)P_3 phosphatase. Nevertheless, the data in Table 4 may explain how Li$^+$, by potentiating an accumulation of Ins(1,4)P_2 in stimulated cells, thereby increases the concentration of Ins(1,3,4)P_3 more significantly than that of Ins(1,4,5)P_3 (Burgess *et al.*, 1985; Hansen *et al.*, 1986).

Table 4. Effect of Li$^+$ and Ins(1,4)P_2 on InsP_3 hydrolysis

Incubations (0.5 ml) contained 25 μl of 30% (w/v) liver homogenate. Where Li$^+$ was absent, total K$^+$ was 120 mM, since [KCl] was increased from 30 mM (as in other experiments) to 50 mM, without significant effect on the rate of InsP_3 hydrolysis. Where Li$^+$ (as LiCl) was added, KCl was removed to maintain a constant ionic strength. Ins(1,4)P_2 was included where indicated. Incubations also contained either [4,5-^{32}P]Ins(1,4,5)P_3 or [^{3}H]Ins(1,3,4)P_3 and were terminated after 5 or 20 min respectively. Data are means ± S.E.M. for the numbers of experiments in parentheses: *$P < 0.02$ (paired t test) and **$P < 0.001$ (unpaired t test) compared with controls in the absence of Li$^+$ or Ins(1,4)P_2.

Concn. in incubation (mM)			Substrate hydrolysed (%)	
K$^+$	Li$^+$	Ins(1,4)P_2	Ins(1,4,5)P_3	Ins(1,3,4)P_3
120	0	0	59±8 (4)	66±8 (5)
120	0	0.035	65 (2)	69 (2)
120	0	0.07	48±5 (4)	49±12 (3)
70	50	0	56±7 (4)	61±7 (5)
70	50	0.07	44±8 (4)*	14±2 (3)**

Concluding comments

Ins(1,4,5)P_3 is an intracellular messenger, and it is to be expected that its metabolism, like that of cyclic AMP (Heyworth *et al.*, 1983), will be subject to physiological control. An understanding of such control will require a knowledge both of the enzymes responsible and of their susceptibility to regulation. The activity of the kinase which converts Ins(1,4,5)P_3 into Ins(1,3,4,5)P_4 was inhibited 2–3-fold as [Ca^{2+}] was changed from 0.1 to 10 μM (Irvine *et al.*, 1986*a*). Ins(1,4,5)P_3 is also metabolized by removal of the 5-phosphate (see above, and the Introduction) and, at least in liver, we have been unable to show regulation of this reaction (Table 2). However, our results (see above) and those of others (see the Introduction) emphasize that cells possess cytosolic and particulate forms of Ins(1,4,5)P_3 5-phosphatase. An analogy with the multiple forms of cyclic AMP phosphodiesterase and their diverse patterns of regulation (see, e.g., Heyworth *et al.*, 1983) suggests that

regulation of Ins(1,4,5)P_3 5-phosphatase could still remain to be discovered.

It is not yet clear why cells convert Ins(1,4,5)P_3 into Ins(1,3,4,5)P_3 and then into Ins(1,3,4)P_3 (Irvine *et al.*, 1984, 1985, 1986*a*; Batty *et al.*, 1985; Downes *et al.*, 1986; Hansen *et al.*, 1986). However, this pathway contributes to Ins(1,4,5)P_3 removal in stimulated cells, and it is also possible that Ins(1,3,4,5)P_4 and/or Ins(1,3,4)P_3 will have some as yet undetected function. Li^+ potentiates the receptor-activated accumulation of Ins(1,3,4)P_3, but not of Ins(1,4,5)P_3 or Ins(1,3,4,5)P_4 (Burgess *et al.*, 1984; Hansen *et al.*, 1986). As a result, Li^+ might selectively potentiate any activity of Ins(1,3,4)P_3.

Other workers have assumed that Ins(1,3,4)P_3 is dephosphorylated at the 4-phosphate to yield Ins(1,3)P_2 (Turk *et al.*, 1986; Hansen *et al.*, 1986), possibly through the action of the Li^+-sensitive 4-phosphatase that attacks Ins(1,4,)P_2 (Storey *et al.*, 1984). Our experiments suggest, at least for liver, that this conclusion is incorrect; the major, and maybe the only, route of Ins(1,3,4)P_3 degradation is by the action of a 1-phosphatase. This specific attack on a lone phosphate differs from the dephosphorylation of the other inositol polyphosphates [Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4], where the attacked phosphate is one of a vicinal pair or trio. The Li^+-insensitivity of the largely cytosolic Ins(1,3,4)P_3 1-phosphatase indicates that this enzyme is different from the well-known Li^+-sensitive Ins1*P* 1-phosphatase. It is possible that the 1-phosphate of Ins(1,3,4)P_3, but not that of Ins(1,4,5)P_3, might be removed by the cytosolic and Li^+-insensitive Ins(1,4)P_2 1-phosphatase (Storey *et al.*, 1984; A. J. Morris, D. J. Storey & R. H. Michell, unpublished work). If this was the case, Li^+ inhibition of Ins(1,4)P_2 4-phosphatase would cause an accumulation of Ins(1,4)P_2 that might compete with Ins(1,3,4)P_3 for the 1-phosphatase. This possibility remains to be investigated, but it would fit well with our observations of potent inhibition of Ins(1,3,4)P_3 1-phosphatase activity by a combination of Li^+ and Ins(1,4)P_2, but not by either substance alone.

The more we learn of inositol polyphosphate metabolism in mammalian cells, the more complex becomes the family of enzymes involved in the many observed reactions (Michell, 1986). It must be assumed that this has, for reasons which we are only beginning to understand, been an evolutionarily worthwhile investment of genomic capacity and protein synthesis. Since many of the inositol polyphosphates are only subtly different, we may expect that many of these enzymes will be structurally and evolutionarily related.

Note added in proof (received 9 December 1986)

Recent work has indicated that Ins(1,3,4)P_3 mobilizes intracellular Ca^{2+} in Swiss-mouse 3T3 cells (Irvine *et al.*, 1986*b*) whereas Ins(1,3,4,5)P_4 facilitates Ca^{2+} influx into sea-urchin eggs (Irvine & Moor, 1986). It is not yet known if Ins(1,3,4)P_3 or Ins(1,3,4,5)P_4 stimulate Ca^{2+} fluxes in liver.

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