The dephosphorylation of inositol 1,4-bisphosphate to inositol in liver and brain involves two distinct Li^+ -sensitive enzymes and proceeds via inositol 4-phosphate

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1. Hydrolysis of both enantiomers of inositol 1-phosphate and both enantiomers of inositol 4-phosphate to inositol is inhibited by LiCl in liver and brain. 2. The phosphatase activity is predominantly soluble. 3. Inositol 1,4-bisphosphate is also hydrolysed by the soluble fraction of liver and brain. 4. Bisphosphatase activity is inhibited by LiCl , but is less sensitive than monophosphatase activity. 5. The product of bisphosphatase in liver and brain is inositol 4-phosphate.

INTRODUCTION

It is well established that the receptor-mediated action of phospholipase C on phosphatidylinositol 4,5-bisphosphate gives rise to the second messenger, *myo*-inositol 1,4,5-trisphosphate [$\text{Ins}(1,4,5)\text{P}_3$], which causes release of Ca^{2+} from intracellular stores [1–3]. Removal of the $\text{Ins}(1,4,5)\text{P}_3$ is carried out by two pathways; phosphorylation to inositol 1,3,4,5-tetrakisphosphate [$\text{Ins}(1,3,4,5)\text{P}_4$] [4–8] or dephosphorylation to inositol 1,4-bisphosphate [$\text{Ins}(1,4)\text{P}_2$] [9–13]. This is in turn sequentially dephosphorylated to inositol, as is the $\text{Ins}(1,3,4,5)\text{P}_4$, for resynthesis of phosphatidylinositol (PtdIns) [12–18]. The demonstration of inositol-1-phosphatase [$\text{Ins}(1)\text{Pase}$] activity in brain [14,15] and the dramatic increase in $\text{Ins}(1)\text{P}$ in agonist-stimulated cells treated with Li^+ , an inhibitor of $\text{Ins}(1)\text{Pase}$ [19–21], has led to the widespread acceptance of the idea that $\text{Ins}(1)\text{P}$ is a major product of the dephosphorylation of the inositol polyphosphates. However, lower concentrations of $\text{Ins}(4)\text{P}$ are also found, for example in brain [20] or in platelets [21], and respond in a similar fashion to agonists and Li^+ treatment. In liver, Michell and co-workers [12,13] reported the existence of two separate pathways for $\text{Ins}(1,4)\text{P}_2$ breakdown, one via $\text{Ins}(1)\text{P}$ and the other via $\text{Ins}(4)\text{P}$. The two steps of the latter pathway were insensitive to Li^+ , and the $\text{Ins}(4)\text{Pase}$ was particulate. More recent reports, however, have suggested that, in brain at least, $\text{Ins}(1,4)\text{P}_2$ dephosphorylation is almost completely Li^+ -sensitive [16,17] and proceeds mainly through $\text{Ins}(4)\text{P}$ [17,18]. Moreover, $\text{Ins}(4)\text{Pase}$ in brain was reported to be soluble and very sensitive to Li^+ [17]. A similar conclusion has been reached for intact adrenal glomerulosa cells, where it has been proposed that second-messenger dephosphorylation proceeds through $\text{Ins}(4)\text{P}$ [22] and that $\text{Ins}(1)\text{P}$ is produced mainly by phospholipase C action on PtdIns [23,24].

The recent availability of synthetic $\text{Ins}(1)\text{P}$ and $\text{Ins}(4)\text{P}$ [25] has enabled us to investigate these apparent

discrepancies in more detail. We report that $\text{Ins}(4)\text{P}$ is hydrolysed by a cytosolic Li^+ -sensitive inositol monophosphatase in brain or liver. The same enzyme is responsible for $\text{Ins}(1)\text{P}$ hydrolysis. $\text{Ins}(1,4)\text{P}_2$ is dephosphorylated to $\text{Ins}(4)\text{P}$ by a second cytosolic enzyme, which is also inhibited by Li^+ , but only at higher concentrations. A preliminary report of some of these findings has been presented elsewhere [26].

MATERIALS AND METHODS

Substrates

Inositol monophosphates were synthesized as described by Billington *et al.* [25], DL- $\text{Ins}(1,4)\text{P}_2$ and DL- $\text{Ins}(3,4)\text{P}_2$ as described by Angyal & Tate [27], and $\text{Ins}(1,3)\text{P}_2$ was synthesized by the method of Billington & Baker [28]. D- $[\text{Ins}-2\text{-}^3\text{H}(\text{n})]\text{Ins}(1,4)\text{P}_2$ (1.5 Ci/mmol) was obtained from New England Nuclear (Du Pont, Stevenage, Herts., U.K.) and L- $[\text{U}-^{14}\text{C}]\text{Ins}(1)\text{P}$ (55 mCi/mmol) from Amersham International (Amersham, Bucks., U.K.).

Preparation of tissue homogenates, supernatants and membranes

Finely chopped rat brain, rat liver or bovine brain was mixed with 2 ml of homogenization buffer (150 mM-KCl, 50 mM-Tris/HCl, pH 8.5, 0.1 mM-EGTA, 0.5 mM-EDTA at 4 °C) per g wet wt. of tissue and homogenized at 4 °C with a motor-driven Teflon/glass homogenizer (200 rev./min until tissue dispersed, then at 1000 rev./min for 2 min). Supernatant fractions were obtained by centrifugation of homogenates at 100 000 g for 1 h at 4 °C. The supernatant was centrifuged a second time before use. For preparation of washed membranes, the pellet from the first centrifugation was taken up in homogenization buffer (final volume of 8 times the original volume of homogenate). The suspension was re-homogenized (1000 rev./min for 1 min) and centrifuged as above. This

Abbreviations used: $\text{Ins}(1)\text{P}$, *myo*-inositol 1-phosphate [similarly $\text{Ins}(4)\text{P}$]; $\text{Ins}(1,4)\text{P}_2$, *myo*-inositol 1,4-bisphosphate; $\text{Ins}(1,4,5)\text{P}_3$, *myo*-inositol 1,4,5-trisphosphate [similarly $\text{Ins}(1,3,4)\text{P}_3$]; $\text{Ins}(1,3,4,5)\text{P}_4$, *myo*-inositol 1,3,4,5-tetrakisphosphate; $\text{Ins}(1)\text{Pase}$, *myo*-inositol-1-phosphatase [similarly $\text{Ins}(4)\text{Pase}$, $\text{Ins}(1,4)\text{P}_2\text{ase}$]; PtdIns, phosphatidylinositol.

was repeated four times. The pellet was finally resuspended to twice the original volume of homogenate.

For some P_i -release assays, particularly when the rate of substrate hydrolysis was low, homogenates and supernatants were dialysed in EDTA-washed tubing against 100 vol. of homogenization buffer for 18 h at 4 °C. In some experiments, homogenates were prepared in 0.25 M-sucrose/5 mM-Tris/HCl, pH 8.0 [17].

P_i -release assay

Tissue extracts were incubated at 37 °C in a final volume of 0.3 ml containing 0.1 mM substrate, 250 mM-KCl, 50 mM-Tris/HCl, pH 8.0, and 3 mM-MgCl₂ for periods of time between 15 min and 1 h. Rates were linear for conversion of up to one-third of the substrate, and conditions were adjusted accordingly. Released P_i was measured colorimetrically by the method of Itaya & Ui [29]. Assays were also carried out in the 'physiological' buffer of Storey *et al.* [12], which contained 10 mM-NaCl, 70 mM-potassium glutamate, 30 mM-KCl, 4 mM-MgSO₄, 1 mM-EGTA and 10 mM-Hepes/KOH, pH 7.2.

Radiochemical assay of Ins(1)Pase

Incubations were carried out as described for the P_i -release assay, except that 12.5 nCi of L-[¹⁴C]Ins(1)P was included. Reaction was terminated by the addition of 50 μ l of 10 % (w/v) trichloroacetic acid. The mixture was diluted with 5 ml of water and applied to a column (0.5 ml packed vol.) of Dowex AG1 X8 (formate form) equilibrated with water. The column was washed with a further 5 ml of water, and the radioactivity of the combined eluates (containing free inositol) was determined by scintillation counting. Activities determined by this assay were very similar to those determined by P_i release.

Radiochemical assay of Ins(1,4)P₂ase

Conditions were exactly as for the radiochemical assay of Ins(1)Pase, except that 23 nCi of D-[³H]Ins(1,4)P₂ was used as label unless otherwise indicated. After elution of free inositol from the Dowex column, inositol monophosphates were eluted with 10 ml of 150 mM-ammonium formate/5 mM-Na₂B₄O₇. Radioactivity was determined by scintillation counting.

H.p.l.c. analysis of inositol monophosphates

Reaction mixtures from the radiochemical assay of Ins(1,4)P₂ase were made up on three times the usual scale. The reaction was stopped by addition of 0.2 ml of 10 % trichloroacetic acid. L-[¹⁴C]Ins(1)P (25 nCi) was added as a marker and, in experiments lacking unlabelled Ins(1,4)P₂ as substrate, 50 nmol of DL-Ins(1)P and 50 nmol of DL-Ins(4)P were added as carriers. The mixture was diluted 5-fold with water and applied to 0.5 ml packed vol. of Dowex AG1 X8 (acetate form) equilibrated with water. The column was washed with 5 ml of water, and the monophosphate products and unused Ins(1,4)P₂ were eluted with 4 ml of 1 M-lithium acetate. The eluate was freeze-dried and dissolved in 5 ml of anhydrous ethanol. The solution was passed through a 0.22 μ m-pore-size filter, which retained the insoluble lithium salts of mono- and bis-phosphates. The filter was washed with 1 ml of ethanol, and the inositol phosphates were eluted with 1 ml of water. This desalting procedure was adapted from the method of Burgess *et al.* [30], and gave recoveries of approx. 90 %. The solution was freeze-

dried and taken up in 50 μ l of water, and a 40 μ l sample was subjected to h.p.l.c. on a Spherisorb propylamino column (250 mm $\times$ 4.6 mm i.d.; 5 μ m particle size). The mobile phase was 200 mM-ammonium acetate adjusted to pH 4.0 with acetic acid. Flow rate was 1 ml/min. Fractions (0.5 ml) were collected and counted for ¹⁴C and ³H radioactivity. The positions of elution of Ins(1)P and Ins(4)P were determined by refractive index.

RESULTS

Hydrolysis of Ins(1)P and Ins(4)P

The rates of hydrolysis of inositol monophosphates by homogenates and high-speed supernatants from bovine and rat tissues are shown in Table 1. The P_i -release assay is difficult to use with homogenates, even after dialysis, because of the very high blanks encountered. Some of the synthetic substrates also contain a considerable concentration of free P_i , which interferes with the assay. With these points in mind, we can conclude firstly that monophosphatase activity is predominantly soluble in liver and brain, regardless of the isomer or enantiomer hydrolysed. Secondly, 0.1 mM-DL-Ins(4)P is hydrolysed somewhat faster than 0.1 mM-DL-Ins(1)P, particularly in liver. Of the enantiomers of Ins(1)P, D-Ins(1)P is hydrolysed less fast than the L-enantiomer, as previously found by Hallcher & Sherman [14], but there was no significant difference between the rates of hydrolysis of (+)-Ins(4)P and (–)-Ins(4)P, whose absolute stereochemistry is as yet unknown.

The Ins(1)Pase and Ins(4)Pase activities of supernatant fractions were almost completely inhibited by LiCl. As shown in Fig. 1 for rat brain supernatant, Ins(4)Pase was apparently more sensitive to Li⁺ than was Ins(1)Pase [17], and this was also found for the liver activities. This differential sensitivity to Li⁺ can be readily explained by the uncompetitive nature of the inhibition, and need not imply that two different enzymes are responsible for hydrolysis of the monophosphate isomers. The concentrations of Li⁺ required for 50 % inhibition of both activities were very similar for bovine brain, rat brain and rat liver [1.4 mM, 1.0 mM and 1.0 mM respectively for Ins(1)Pase; 0.33 mM, 0.14 mM and 0.11 mM respectively for Ins(4)Pase].

Although these results agree in general terms with those of Ackermann *et al.* [17], the finding of rapid, Li⁺-sensitive, Ins(4)Pase activity in liver cytosol is not in agreement with Michell and co-workers [12,13]. Since we used much higher concentrations of substrates, especially Ins(4)P, we considered that there may have been a low- K_m low- V_{max} enzyme of quite different properties, which was masked in our experiments but dominant in those of Storey *et al.* [12]. With L-[¹⁴C]Ins(1)P diluted with unlabelled DL-Ins(1)P as substrate for the radiochemical assay, activity in rat brain supernatant insensitive to 200 mM-LiCl increased from 3 % to only 6.6 % as the substrate concentration was lowered from 100 μ M to 1 μ M. Decreased sensitivity would be expected in any case for an uncompetitive inhibitor, as described in more detail below. We therefore conclude that the same enzyme is responsible for hydrolysis of Ins(1)P at high or low substrate concentration. Unfortunately, the unavailability of labelled Ins(4)P prevented us from carrying out the same type of experiment with this isomer. However, we were able to reach the same conclusion for Ins(4)P

Table 1. Inositol monophosphatase activities in liver and brain

Activities were determined by using the P_i -release assay, and are given as nmol ($\pm$ S.E.M.)/min per ml of fraction; nd, not determined. Numbers of determinations are given in parentheses.

Substrate	Bovine brain		Rat brain		Rat liver	
	Homogenate	Supernatant	Homogenate	Supernatant	Homogenate	Supernatant
DL-Ins(1) <i>P</i>	60 $\pm$ 5 (5)	61 $\pm$ 4 (8)	63	52 $\pm$ 7 (3)	34 $\pm$ 3 (3)	36 $\pm$ 3 (5)
L-Ins(1) <i>P</i>	70 $\pm$ 11 (3)	80 $\pm$ 6 (4)	nd	nd	nd	nd
D-Ins(1) <i>P</i>	64 $\pm$ 3 (3)	61 $\pm$ 8 (4)	nd	nd	nd	nd
DL-Ins(4) <i>P</i>	64 $\pm$ 9 (3)	84 $\pm$ 4 (4)	nd	80 $\pm$ 2 (2)	70	70 $\pm$ 5 (3)
(-)-Ins(4) <i>P</i>	42 $\pm$ 5 (3)	63 $\pm$ 9 (3)	nd	nd	nd	nd
(+)-Ins(4) <i>P</i>	59 $\pm$ 12 (3)	67 $\pm$ 8 (4)	nd	nd	nd	nd

Fig. 1. Li^+ inhibition of monophosphatase activity

Activity was measured by the P_i -release assay in rat brain supernatant with DL-Ins(1)*P* (●) or DL-Ins(4)*P* (▲). Incubations for 15 min were carried out as described in the Materials and methods section, with 10 μ l of supernatant fraction.

Fig. 2. Li^+ -sensitivity of Ins(1)*P*ase activity in tissue homogenates

Activity was measured by the radiochemical assay in rat liver (○), rat brain (●) and bovine brain (▲). Homogenates (10 μ l) were incubated for either 15 min (brain) or 25 min (liver).

from experiments described below on the hydrolysis of Ins(1,4) P_2 .

In tissue homogenates, approx. 20 % of the Ins(1)*P*ase activity (with 0.1 mM substrate) was insensitive to 100 mM- Li^+ (Fig. 2). Washed membranes from bovine brain contained 7 % of the original homogenate activity, but this was nearly completely inhibited by 100 mM- Li^+ . The Li^+ -sensitive Ins(1)*P*ase therefore appears to be membrane-associated to a small extent, whereas the Li^+ -insensitive activity is either removed from the membranes by the washing procedure or inactivated. In support of the former conclusion, we have found that the supernatant fraction contains a low amount of Li^+ -insensitive Ins(1)*P*ase activity, which can be separated from the Li^+ -sensitive enzyme by anion-exchange chromatography (results not shown).

The difficulty of measuring low Ins(4)*P*ase activity in homogenates by the P_i -release assay prevented us from obtaining a precise value for Li^+ -insensitive Ins(4)*P*ase by this method. However, using radiolabelled Ins(1,4) P_2 to generate the Ins(4)*P*, we could show that the Li^+ -insensitive activity in homogenates is very low, as described in detail below.

Hydrolysis of inositol bisphosphates

Rates of hydrolysis of Ins(1,4) P_2 , Ins(3,4) P_2 and Ins(1,3) P_2 are shown in Table 2. By far the most rapidly hydrolysed is Ins(1,4) P_2 and, in brain or liver, the enzyme is predominantly soluble. The other bisphosphates are potential hydrolysis products of Ins(1,3,4) P_3 produced from Ins(1,3,4,5) P_4 . Although Hansen *et al.* [6] tentatively concluded that Ins(1,3) P_2 is the major product, more convincing evidence has now identified Ins(3,4) P_2 as the bisphosphate produced from Ins(1,3,4) P_3 [18,31]. Despite this, Ins(3,4) P_2 is only very slowly hydrolysed by bovine brain and by rat liver. Ins(1,3) P_2 , on the other hand, is hydrolysed at faster rates, particularly by liver supernatant. These low activities prevented accurate measurements being made with tissue homogenates, but rates were certainly not higher than with supernatants, indicating that these other bisphosphates are hydrolysed by soluble enzymes. The potential products of hydrolysis of these bisphosphates are Ins(1)*P*, Ins(4)*P* and Ins(3)*P*. However, D- and L-Ins(3)*P* are identical with L- and D-Ins(1)*P* respectively, so that all products will be substrates for the rapid Li^+ -sensitive Ins(1)*P*ase or Ins(4)*P*ase. Since rates were measured by P_i release, further hydrolysis of the monophosphate products must have contributed

Table 2. Inositol bisphosphatase activities in liver and brain

Activities were determined by using the P_i -release assay as in Table 1. Incomplete measurements suggest that rat brain activities are closer to those of rat liver than of bovine brain. Numbers of determinations are given in parentheses; nd, not determined.

Substrate	Bovine brain		Rat liver	
	Homogenate	Supernatant	Homogenate	Supernatant
DL-Ins(1,4) P_2	44 ± 5 (3)	46 ± 9 (7)	53 ± 2 (2)	64 ± 10 (2)
DL-Ins(3,4) P_2	nd	2 ± 1 (3)	nd	10 ± 3 (3)
Ins(1,3) P_2	nd	4 ± 1 (3)	nd	23 ± 2 (3)

Fig. 3. Hydrolysis of 0.1 mM-Ins(1,4) P_2 by rat brain supernatant

Reaction was carried out for 15 min with 10 μ l of supernatant fraction. Reaction mixtures were analysed on Dowex columns (see the Materials and methods section) for [3 H]inositol (○) and [3 H]inositol monophosphate (▲). The sum (●) indicates the amount of Ins(1,4) P_2 hydrolysed.

to the apparent bisphosphatase rates. For Ins(1,4) P_2 , this point is considered in more detail below, but, in view of the slow hydrolysis of Ins(1,3) P_2 and Ins(3,4) P_2 , it is probable that the measured rates in the P_i -release assay for these substrates include both phosphate residues.

The availability of D-[3 H]Ins(1,4) P_2 enabled us to investigate the hydrolysis of this bisphosphate in greater detail by the radiochemical assay. As shown in Fig. 3, when 0.1 mM-DL-Ins(1,4) P_2 containing D-[3 H]Ins(1,4) P_2 was incubated with rat brain supernatant for a length of time which allowed conversion of approximately half of the substrate, labelled inositol monophosphate(s) and labelled inositol derived from monophosphate hydrolysis were found. The latter reaction was inhibited by low concentrations of Li^+ which left Ins(1,4) P_2 hydrolysis unaffected. At higher Li^+ concentrations, however, Ins(1,4) P_2 ase was also inhibited, producing a bell-shaped curve for the dependence of monophosphate concentration on Li^+ concentration. Ins(1,4) P_2 ase was more than 90% inhibited by 100 mM-LiCl. Results obtained with rat liver supernatant or with homogenates from

Fig. 4. Hydrolysis of 0.1 mM-Ins(1,4) P_2 by rat liver homogenate

Conditions were as in the legend to Fig. 3. ○, [3 H]inositol; ▲, [3 H]inositol monophosphate; ●, the sum indicates the amount of Ins(1,4) P_2 hydrolysed.

Fig. 5. H.p.l.c. analysis of the product of Ins(1,4) P_2 ase

Products of hydrolysis of 0.1 mM-[3 H]Ins(1,4) P_2 by rat brain supernatant were analysed by h.p.l.c. ●, [3 H]-Inositol monophosphate; ▲, [14 C]Ins(1) P marker.

either tissue (Fig. 4) were very similar, indicating that Ins(1,4) P_2 ase activity is cytosolic and Li^+ -sensitive in both liver and brain. Substantial inhibition by Li^+ of D-Ins(1,4) P_2 hydrolysis by rat brain supernatant has also been found by Delvaux *et al.* [16] and Ackermann *et al.* [17]. In contrast with the latter group's findings, and with

the conclusions of Storey *et al.* [12], analysis of the monophosphate products by h.p.l.c. revealed that by far the major product was Ins(4)P, with only traces (< 2%) of Ins(1)P (Fig. 5). The same result was encountered in the presence or the absence of Li⁺ (up to 100 mM) in liver as well as brain, and with homogenates or supernatants.

Ackermann *et al.* [17] found that Ins(1)P accounted for 25% of the monophosphate product from hydrolysis of D-Ins(1,4)P₂ by rat brain supernatant, although, with a partially purified monophosphatase preparation, hydrolysis of DL-Ins(1,4)P₂ resulted in a substantially higher proportion of Ins(4)P. Moreover, in liver, Storey *et al.* [12] concluded that Ins(1,4)P₂ hydrolysis via Ins(4)P was Li⁺-insensitive. We considered several possibilities for the disparity between these results. Firstly, adopting the homogenization medium of Storey *et al.* [12] and Ackermann *et al.* [17] or the former group's 'physiological' incubation buffer made no difference to the relative amounts of monophosphate products. Secondly, we considered the possibility that our use of unlabelled DL-Ins(1,4)P₂ with radiolabelled D-Ins(1,4)P₂ was responsible. Certainly, since very little [³H]Ins(1)P was detected, D-Ins(1)P was not being produced, but it was possible that the L enantiomer of the bisphosphate or its hydrolysis product was an inhibitor of Ins(1)P production by a second enzyme. To check this, we analysed the products of hydrolysis of D-[³H]Ins(1,4)P₂ in the absence of added DL-Ins(1,4)P₂. Again, Ins(4)P was produced, with only a trace of Ins(1)P.

The last significant difference between our experiments and those of others lay in the concentrations of substrates used. Whereas Ackermann *et al.* [17] used 0.7 mM-Ins(1,4)P₂ routinely, we have used 0.1 mM, since this is close to the *K_m* for bisphosphate and within the range claimed for rat liver and brain (50–150 mmol/kg of tissue; [32]). Although this difference is not very great, Storey *et al.* [12] used between 40 and 300 nM-D-Ins(1,4)P₂ as substrate in their experiments with rat liver. We have therefore carried out experiments similar to that of Fig. 1, but at much lower substrate concentrations. Fig. 6 shows the result of incubating rat brain supernatant with 22 nM-D-Ins(1,4)P₂. Although Ins(4)P was still 98% of the total monophosphate product, the most striking change was the insensitivity of bisphosphatase and decreased sensitivity of monophosphatase to LiCl, in agreement with the results of Storey *et al.* [12]. However, this difference can be accounted for by the uncompetitive nature of Li⁺ inhibition, without the need to postulate different enzymes operating under these conditions. Bovine brain monophosphatase has a *K_m* for DL-Ins(4)P of 0.23 mM and a *K_i* for Li⁺ of 0.26 mM [33]. Thus with, say, 20 nM-Ins(4)P, one would expect 50% inhibition with 1.5 M-Li⁺ if Li⁺ acted purely uncompetitively. The observation that inhibition is observed at 10–100 mM-Li⁺ suggests that Li⁺ also binds weakly to the free enzyme, with a *K_i* of approx. 10 mM. As the substrate concentration is decreased, the Li⁺ concentration required for 50% inhibition will increase to a limiting value of 10 mM (Fig. 6). The Ins(1,4)P₂ase is also uncompetitively inhibited by Li⁺ (*K_i* in bovine brain is 8 mM), so loss of sensitivity at low concentration of substrate is also to be expected. Nevertheless, we could not exclude, as an alternative explanation, the existence of a second Ins(4)Pase of low *K_m*, low *V_{max}*, and relatively poor Li⁺ sensitivity. Since competitive inhibitors become more potent at lower substrate concentrations, we

Fig. 6. Hydrolysis of 22 nM-Ins(1,4)P₂ by rat brain supernatant

Conditions were otherwise as in the legend to Fig. 3. ○, [³H]Inositol; ▲, [³H]inositol monophosphate; ●, the sum indicates the amount of Ins(1,4)P₂ hydrolysed.

looked at the effects of 2'-AMP, a substrate for monophosphatase [15], on the hydrolysis products of Ins(1,4)P₂. Complete inhibition of [³H]inositol production was obtained regardless of the substrate concentration, and the concentrations required for 50% inhibition were 0.9 mM and 0.6 mM for 0.1 mM and 22 nM substrate respectively. The increased sensitivity to 2'-AMP at lower substrate favours our view that the same enzyme is responsible for inositol production at any substrate concentration.

DISCUSSION

We conclude from this work that Ins(1,4)P₂ hydrolysis in brain or liver is carried out by a cytosolic Li⁺-sensitive activity which removes the 1-phosphate, in agreement with the work of Inhorn *et al.* [18]. Ins(1)P is produced only in very small amounts. The resulting inositol monophosphates are hydrolysed by a second cytosolic Li⁺-sensitive activity to inositol. On the basis of the present work alone, we cannot decide how many enzymes may be responsible for these reactions. Ackermann *et al.* [17] suggested that bovine brain Ins(1)Pase hydrolyses both Ins(1)P and Ins(4)P, but the preparation of enzyme they used was less than 2% pure, on the basis of its specific activity. Nevertheless, their conclusion was correct, since we have purified the bovine brain enzyme to homogeneity and shown that it hydrolyses both enantiomers of Ins(1)P and both enantiomers of Ins(4)P [33].

Although the uncompetitive nature of Li⁺ inhibition goes some way towards explaining the results of Storey *et al.* [12], we cannot fully account for their observation of specific Ins(1)Pase and Ins(4)Pase activities, the high proportion of Ins(1)P produced, and the separation of Ins(1,4)P₂ 1- and 4-phosphatase activities by ion exchange [13]. However, the demonstration by us and others [17] of Li⁺-sensitive cytosolic Ins(4)Pase means that the release of [³²P]P_i from Ins(1,4)P₂ cannot be unambiguously interpreted as being due to Ins(1,4)P₂-4-phosphatase activity, since [³²P]P_i could arise from

Ins(1,4) P_2 -1-phosphatase followed by the subsequent hydrolysis of the Ins([32 P]4) P to inositol. The inhibition of the putative Ins(1,4) P_2 -4-phosphatase by Li^+ (50% with 2.5 mM) and its co-chromatography with monophosphatase on gel filtration and ion-exchange chromatography support this viewpoint. Michell and co-workers [13] even considered the possibility that Ins(1) P ase and Ins(1,4) P_2 -4-phosphatase might be the same enzyme, but dismissed this idea because of the greater heat-lability of the Ins(1,4) P_2 -4-phosphatase. However, this becomes understandable if the latter activity is in reality due to the combined action of a heat-labile Ins(1,4) P_2 -1-phosphatase and a Li^+ -sensitive Ins(4) P ase.

Our conclusions lend further support to ideas that the increase in Ins(1) P encountered in Li^+ -treated and agonist-stimulated cells and tissues arises primarily, if not exclusively, from the action of phospholipase C on PtdIns [23,24]. As shown by Inhorn *et al.* [18], breakdown of Ins(1,3,4,5) P_4 via Ins(3,4) P_2 gives rise to D-Ins(3) P [the same as L-Ins(1) P] by the action of a 4-phosphatase. However, this cannot account for the increase in Ins(1) P , since it is the D-isomer which is primarily increased [19]. Similarly, we can exclude Ins(1) P production from glucose 6-phosphate, since this reaction produces the L enantiomer [34]. Although the increased hydrolysis of PtdIns enables us to explain how increase in Ins(1) P can occur, even though it is not an intermediate of second-messenger degradation, we still have no idea as to what function increased PtdIns metabolism serves.

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Received 7 May 1987/7 August 1987; accepted 10 September 1987