Enzymic dephosphorylation of D-*myo*-inositol 1,4-bisphosphate in rat brain

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Inositol 1,4,5-trisphosphate [$\text{Ins}(1,4,5)\text{P}_3$] and inositol 1,4-bisphosphate [$\text{Ins}(1,4)\text{P}_2$] phosphatase activities were measured in both 180 000 g (60 min) particulate and supernatant fractions of rat brain homogenates. Although $\text{Ins}(1,4,5)\text{P}_3$ was mostly hydrolysed by a particulate phosphatase [Erneux, Delvaux, Moreau & Dumont (1986) *Biochem. Biophys. Res. Commun.* **134**, 351–358], $\text{Ins}(1,4)\text{P}_2$ phosphatase was predominantly soluble. The latter enzyme was Mg^{2+} -dependent and sensitive to thiol-blocking agents (e.g. *p*-hydroxymercuribenzoate). In contrast with $\text{Ins}(1,4,5)\text{P}_3$ phosphatase activity measured in the soluble fraction, $\text{Ins}(1,4)\text{P}_2$ phosphatase was insensitive to 0.001–1 mM-2,3-bisphosphoglycerate. Lithium salts, widely used in psychiatric treatment, inhibited both $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1)\text{P}_1$ phosphatase activities of the crude soluble fraction. In particular, 50% inhibition of phosphatase activity, with 2 μM - $\text{Ins}(1,4)\text{P}_2$ as substrate, was achieved at 3–5 mM-LiCl. At these concentrations, LiCl did not change $\text{Ins}(1,4,5)\text{P}_3$ phosphatase activity measured in the same fraction with 1–4 μM - $\text{Ins}(1,4,5)\text{P}_3$ as substrate. Chromatography of the soluble fraction of a rat brain homogenate on DEAE-cellulose resolved three phosphatase activities. These forms, peaks I, II and III, dephosphorylated $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1)\text{P}_1$ and $\text{Ins}(1,4)\text{P}_2$ respectively. If LiCl (10 mM) was included in the assay mixture, it inhibited both peak-II $\text{Ins}(1)\text{P}_1$ phosphatase and peak-III $\text{Ins}(1,4)\text{P}_2$ phosphatase, suggesting the existence of at least two Li^+ -sensitive phosphatases.

INTRODUCTION

The importance of $\text{Ins}(1,4,5)\text{P}_3$ as an intracellular messenger of Ca^{2+} -mobilizing hormones is now well established in many cells, including neuronal tissues (Downes, 1983; Berridge & Irvine, 1984; Michell, 1986). Dephosphorylation of $\text{Ins}(1,4,5)\text{P}_3$ occurs through a cascade of specific phosphatases that sequentially convert $\text{Ins}(1,4,5)\text{P}_3$ into $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1)\text{P}_1$. Both membrane-bound and soluble $\text{Ins}(1,4,5)\text{P}_3$ phosphatase activities have been described in human erythrocytes (Downes *et al.*, 1982) and platelets (Connolly *et al.*, 1985), rat liver (Seyfred *et al.*, 1984; Storey *et al.*, 1984; Joseph & Williams, 1985) and brain (Erneux *et al.*, 1986). Those studies have characterized an inositol 5-phosphatase that converts $\text{Ins}(1,4,5)\text{P}_3$ into $\text{Ins}(1,4)\text{P}_2$ and P_i . $\text{Ins}(1,4)\text{P}_2$ was not a substrate of the purified enzyme from human platelets (Connolly *et al.*, 1985). Little is known about $\text{Ins}(1,4)\text{P}_2$ phosphatase activity, although removal of the 4- and 1-phosphate groups is required for producing *myo*-inositol, the precursor for phosphatidylinositol. Lithium, which is widely used in the treatment of manic-depressive illness, is also a potent inhibitor of $\text{Ins}(1)\text{P}_1$ phosphatase activity of brain tissue (Hallcher & Sherman, 1980; Takimoto *et al.*, 1985). In rat liver, $\text{Ins}(1,4)\text{P}_2$ is degraded by a soluble phosphatase to $\text{Ins}(1)\text{P}_1$ as well as to $\text{Ins}(4)\text{P}_1$ (Storey *et al.*, 1984). That enzyme activity is sensitive to inhibition by Li^+ . It is generally accepted now that, in the presence of Li^+ , intracellular inositol supply is greatly decreased and phosphatidylinositol synthesis inhibited (Downes & Stone, 1986).

The intracellular concentration of $\text{Ins}(1,4,5)\text{P}_3$ is

determined by the rate of its release from phosphatidylinositol 4,5-bisphosphate, the rate of its phosphorylation to $\text{Ins}(1,3,4,5)\text{P}_4$ (Irvine *et al.*, 1986) and the rate of its catabolism by phosphatases. Previous work on cyclic nucleotide phosphodiesterases has demonstrated the importance of the catabolic pathway of cyclic AMP as a target for positive or negative regulation of the cyclic AMP system (Erneux *et al.*, 1985). The system is rather complex, with tissue- or cell-specific enzymes and isoenzymes. The presence (or absence) of those enzymes often explains cell-specificity of phosphodiesterase control. Dephosphorylation reactions of $\text{Ins}(1,4,5)\text{P}_3$ by phosphatase(s) could similarly be a major factor in the regulation of the $\text{Ins}(1,4,5)\text{P}_3$ signal. The purpose of this work is to define further the enzymes involved in this metabolism in rat brain. We show the inhibitory effect of Li^+ on two apparently different phosphatases that dephosphorylate $\text{Ins}(1)\text{P}_1$ and $\text{Ins}(1,4)\text{P}_2$ respectively.

MATERIALS AND METHODS

Materials

[2- ^3H] $\text{Ins}(1)\text{P}_1$ (sp. radioactivity 8 Ci/mmol) and [2- ^3H] $\text{Ins}(1,4)\text{P}_2$ (radioactivity 2 Ci/mmol) were obtained from New England Nuclear Corp.; $\text{H}_3^{32}\text{PO}_4$ (sp. radioactivity 10 mCi/ml; carrier-free) and $\text{Ins}(1)\text{P}_1$ were from Amersham International. 2,3-Bisphosphoglycerate, phenylmethanesulphonyl fluoride and leupeptin were from Sigma (London), *p*-hydroxymercuribenzoate and bovine serum albumin from Serva (Heidelberg, Germany), and Triton X-100 was from Boehringer (Mannheim, Germany). Dowex AG1-X8 (formate form;

Abbreviations used: $\text{Ins}(1)\text{P}_1$, inositol 1-phosphate; $\text{Ins}(4)\text{P}_1$, inositol 4-phosphate; $\text{Ins}(1,4)\text{P}_2$, inositol 1,4-bisphosphate; $\text{Ins}(1,4,5)\text{P}_3$, inositol 1,4,5-trisphosphate; $\text{Ins}(1,3,4,5)\text{P}_4$, inositol 1,3,4,5-tetrakisphosphate.

200–400 mesh) and Dowex AG1-X8 (Cl⁻ form; 200–400 mesh) were from Bio-Rad, Sephadex G-10 was from Pharmacia, and DEAE-cellulose (DE-52) from Whatman. Polyethyleneimine-cellulose t.l.c. plates, LiCl, NaCl, KCl and NH₄Cl were obtained from Merck. The h.p.l.c. column (μ Bondapak NH₂; 30 cm $\times$ 0.39 cm) was purchased from Waters. Human blood was given by the Blood Transfusion Center and antibiotics were obtained from Flow Laboratories.

Preparation of ³²P-labelled substrates

[4-³²P]Ins(1,4)P₂ and [³²P]Ins(1,4,5)P₃ were prepared from ³²P-labelled human erythrocyte membranes by published procedures (Downes *et al.*, 1982; Irvine *et al.*, 1984). In the preparation of [³²P]Ins(1,4)P₂ and [³²P]Ins(1,4,5)P₃ from human erythrocytes, the 1-phosphate remains unlabelled (Downes *et al.*, 1982; Hawkins *et al.*, 1984). We started with about 200 ml of concentrated erythrocytes. Cells were incubated for 13 h at 30 °C in the rejuvenating medium defined by Shukla *et al.* (1978) in the presence of penicillin G and streptomycin (both at 100 μ g/ml) and 20 mCi of H₃³²PO₄. [³²P]Ins(1,4,5)P₃ and [³²P]Ins(1,4)P₂ were isolated by Dowex (formate form) chromatography as described by Downes & Michell (1981). The [³²P]Ins(1,4)P₂ eluate obtained from the first chromatography was separately rechromatographed on a similar Dowex column to remove contaminating [³²P]P_i and [³²P]Ins(1,4,5)P₃. Samples of inositol phosphates were desalted either by Dowex (Cl⁻ form) chromatography, eluted with 1 M-LiCl (Downes *et al.*, 1982), or Sephadex G-10 chromatography (Wilson *et al.*, 1985). Quantitative measurement of phosphate content was performed by the HClO₄ procedure (Ernster *et al.*, 1950). Substrates, [³²P]Ins(1,4)P₂ and [³²P]Ins(1,4,5)P₃, identified on polyethyleneimine-cellulose t.l.c. (Emilsson & Sundler, 1984), were checked to be pure by h.p.l.c. on a μ Bondapak NH₂ column as described by Binder *et al.* (1985). [³²P]Ins(1,4,5)P₃ and [³²P]Ins(1,4)P₂ prepared from human erythrocytes co-migrated with [³H]Ins(1,4,5)P₃ and [³H]Ins(1,4)P₂ (purchased from NEN), respectively, on a μ Bondapak NH₂ column.

Incubation and assay of inositol phosphate phosphatases

The incubation mixture contained the phosphatase in 50 mM-Hepes/NaOH (pH 7.4), 5 mM-2-mercaptoethanol, 1 mg of bovine serum albumin/ml and 1000–3000 c.p.m. of [³²P]Ins(1,4,5)P₃ (1–6 μ M) or [³²P]Ins(1,4)P₂ (1–4 μ M), in 0.1 ml. The reaction was initiated by adding the enzyme, followed by incubation for 1–7 min at 37 °C. The reaction was terminated by adding 0.1 ml of ice-cold 20 % (v/v) trichloroacetic acid. After 15 min, the precipitates were pelleted by centrifugation (30 min, 3000 rev./min) at 4 °C, 0.17 ml of each supernatant was extracted with 3 $\times$ 0.5 ml of diethyl ether to remove the trichloroacetic acid and neutralized with 2 μ l of conc. NH₃. The inositol phosphates were separated on a 0.2 ml Dowex (formate form) column as described by Downes & Michell (1981). Enzyme activities were estimated from the ³²P eluted in the phosphate peak on Dowex (formate form) chromatography. We do not separate, and so do not distinguish between, [³²P]P_i and [³²P]Ins(4)P₁, the potential products of [4-³²P]Ins(1,4)P₂ dephosphorylation. In the phosphatase assay, we are rather measuring a dephosphorylation reaction of [³²P]Ins(1,4)P₂. Ins(1)P₁ phosphatase was measured as

described above, with the same incubation mixture, in which the substrate was 1000–3000 c.p.m. of [³H]Ins(1)P₁ (0.01–5 μ M). The product, [³H]inositol, was eluted with water and radioactivity was measured by liquid-scintillation counting.

Enzyme preparation and DEAE-cellulose chromatography

In general, 3 g of fresh brains from Sprague–Dawley male rats was homogenized at 0–4 °C in 12 ml of the following buffer: 20 mM-Tris/HCl (pH 7.5), 0.4 mM-phenylmethanesulphonyl fluoride, 5 μ M-leupeptin, 0.25 M-sucrose. This preparation was centrifuged at 180 000 g for 1 h. The supernatant was removed and the pellet fraction resuspended in the homogenizing medium (final volume equal to the whole-homogenate volume). About 31 mg of the freshly prepared soluble fraction was applied to a DEAE-cellulose DE-52 column (0.9 cm $\times$ 14 cm) that had been pre-equilibrated with 20 mM-Tris/HCl (pH 7.5)/0.1 mM-EDTA/0.4 mM-phenylmethanesulphonyl fluoride/5 mM-2-mercaptoethanol/10% (v/v) glycerol (buffer A). After application, the column was washed with 100 ml of buffer A, followed by a linear gradient of 0–0.25 M-NaCl (total vol. 400 ml) made in buffer A. Fractions (8–8.5 ml, depending on the profile) were collected.

RESULTS

General properties of Ins(1,4)P₂ dephosphorylation in rat brain

When [³²P]Ins(1,4)P₂ (1–6 μ M) was incubated in the presence of a rat brain homogenate, it was readily dephosphorylated. Ins(1,4)P₂ phosphatase activity was most abundant in the 180 000 g supernatant fraction, suggesting that the activity was predominantly soluble. Some activity remained in the particulate fraction (about 9–10% of the total activity at 2 μ M-[³²P]Ins(1,4)P₂), but, in contrast with Ins(1,4,5)P₃ phosphatase activity (Erneux *et al.*, 1986), Ins(1,4)P₂ phosphatase of the pellet fraction was insensitive to low (0.03%) concentration of Triton X-100 (results not shown). It has been reported that purified rat liver membranes showed an Ins(1,4,5)P₃ phosphatase activity, but no detectable Ins(1,4)P₂ phosphatase activity (Storey *et al.*, 1984; Seyfred *et al.*, 1984; Joseph & Williams, 1985). We repeated those observations with rat liver membranes prepared by the method of Pilkis *et al.* (1974).

Since the 180 000 g rat brain supernatant exhibited the highest specific activity of Ins(1,4)P₂ phosphatase, we used that fraction for further characterization. [³²P]Ins(1,4)P₂ could be totally dephosphorylated by incubation with rat brain soluble fraction. The data shown in Fig. 1(a) are expressed as the proportion of total ³²P released in the phosphate peak on Dowex (formate form) chromatography (see Hawkins *et al.*, 1984). The dephosphorylated products, which are ³²P-labelled, could be either [³²P]P_i or [³²P]Ins(4)P₁, but those co-migrated on Dowex columns used in the phosphatase assay. When short incubation times (2–4 min) were used, Ins(1,4)P₂ phosphatase activities were linear with respect to protein concentration (up to 0.15 mg/ml and in the presence of 10 mM-LiCl in Fig. 1b). In particular, as observed for soluble and particulate

Fig. 1. Inhibition of $\text{Ins}(1,4)\text{P}_2$ dephosphorylation by LiCl

(a) Time course of $[\text{}^{32}\text{P}]\text{Ins}(1,4)\text{P}_2$ dephosphorylation by a rat brain soluble fraction in the presence (○) or absence (●) of 10 mM-LiCl. $[\text{}^{32}\text{P}]\text{Ins}(1,4)\text{P}_2$ concentration was $2\text{ }\mu\text{M}$. Protein concentration was 0.081 mg/ml . Data are means of triplicates ($\pm\text{s.d.}$) and are expressed as the percentage of total ^{32}P released in the phosphate peak by Dowex (formate form) chromatography. Data are representative of three different preparations of $[\text{}^{32}\text{P}]\text{Ins}(1,4)\text{P}_2$. (b) Rat brain soluble $\text{Ins}(1,4)\text{P}_2$ phosphatase activity as a function of protein concentration in the presence (○) or absence (●) of 10 mM-LiCl. The substrate was $[\text{}^{32}\text{P}]\text{Ins}(1,4)\text{P}_2$ at $2.05\text{ }\mu\text{M}$ and the incubation time was 4 min. The data are expressed as pmol/min (means of triplicates $\pm\text{s.d.}$). (c) Dose-response curve of LiCl inhibition of rat brain soluble $\text{Ins}(1,4)\text{P}_2$ phosphatase activity. $[\text{}^{32}\text{P}]\text{Ins}(1,4)\text{P}_2$ was at $2\text{ }\mu\text{M}$, protein concentration was 0.073 mg/ml and the incubation time was 4 min. Data are expressed as the percentage of control value (means of triplicates $\pm\text{s.d.}$). Control activity was measured in the absence of LiCl.

$\text{Ins}(1,4,5)\text{P}_3$ phosphatase activity (Erneux *et al.*, 1986), $\text{Ins}(1,4)\text{P}_2$ dephosphorylation was Mg^{2+} -dependent and sensitive to thiol-blocking agents (e.g. *p*-hydroxymercuribenzoate). As shown in Fig. 2, $50\text{ }\mu\text{M}$ -*p*-hydroxymercuribenzoate totally inactivated phosphatase activities with micromolar concentrations of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,4)\text{P}_2$ as substrates. The effect was much less pronounced for $\text{Ins}(1)\text{P}_1$ phosphatase activity, with $[\text{}^3\text{H}]\text{Ins}(1)\text{P}_1$ substrate at $5\text{ }\mu\text{M}$. 2,3-Bisphosphoglycerate possesses a pair of vicinal phosphate groups and was an inhibitor of the $\text{Ins}(1,4,5)\text{P}_3$ phosphatase of erythrocyte membranes (Downes *et al.*, 1982; results not shown). In our studies, 2,3-bisphosphoglycerate was a potent inhibitor of $\text{Ins}(1,4,5)\text{P}_3$ phosphatase activity of rat brain soluble fraction (total inhibition at 1 mM -2,3-bisphosphoglycerate), but had no inhibitory effect on $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1)\text{P}_1$ phosphatase activities measured in the same fraction (Fig. 3).

Inhibition of $\text{Ins}(1,4)\text{P}_2$ dephosphorylation by LiCl

LiCl inhibited $\text{Ins}(1,4)\text{P}_2$ phosphatase activity of rat brain soluble fraction. A typical concentration-response curve is shown in Fig. 1(c): 50% inhibition occurred at 3–5 mM- Li^+ , at micromolar $\text{Ins}(1,4)\text{P}_2$ substrate concentration. The effect was not produced by NH_4Cl , KCl or NaCl tested in the 1–30 mM range (results not shown). In rat brain, sensitivity to Li^+ was observed in the protein-concentration range $0.02\text{--}0.16\text{ mg/ml}$ (Fig. 1b) and was observed at the shortest incubation time tested, i.e. 1 min (Fig. 1a). The sensitivity to Li^+ remained after DEAE-cellulose chromatography of rat brain soluble fraction (see below).

Separation of activities by DEAE-cellulose chromatography

Since rat brain shows the presence of a Li^+ -sensitive $\text{Ins}(1)\text{P}_1$ phosphatase (Hallcher & Sherman, 1980;

Fig. 2. Inactivation of inositol phosphate phosphatase activities, measured in crude soluble rat brain fraction, by *p*-hydroxymercuribenzoate

Substrates were 5 μM-[³²P]Ins(1,4,5)P₃ (■), 2 μM-[³²P]Ins(1,4)P₂ (●) and 5 μM-[³H]Ins(1)P₁ (▼). Activities are expressed as percentages of that of enzyme incubated in the absence of *p*-hydroxymercuribenzoate (means of triplicates ± S.D.).

Takimoto *et al.*, 1985), we addressed the question of whether the same enzyme could use Ins(1,4)P₂ as substrate and be inhibited by Li⁺. Chromatography of the soluble fraction of rat brain on DEAE-cellulose resolved three phosphatase activities (Fig. 4). Peaks Ia and Ib dephosphorylated only Ins(1,4,5)P₃. Peaks II and III, which were eluted later on the gradient, hydrolysed Ins(1)P₁ and Ins(1,4)P₂ respectively (Fig. 4a). Although the wash fluid (80–100 ml) contained about 70% of the protein of the original supernatant fluid, no measurable inositol phosphatase activities were detected in it. Total phosphatase activity, with any inositol phosphate, recovered from the DEAE-cellulose column amounted to 30–60% of that applied. As reported in rat liver (Storey *et al.*, 1984; Seyfred *et al.*, 1984; Joseph & Williams, 1985) or human platelets (Connolly *et al.*, 1985), Li⁺ up to 5 mM had no effect on Ins(1,4,5)P₃ phosphatase activity in rat brain crude fraction (results not shown). On the contrary, Li⁺ inhibited the dephosphorylation of both Ins(1)P₁ and Ins(1,4)P₂: both peak-II and peak-III phosphatases were separated by ion-exchange chromatography and were Li⁺-sensitive (Fig. 4b).

DISCUSSION

Considerable interest has been focused on Ins(1,4,5)P₃ and 1,2-diacylglycerol, which are formed by receptor-activated breakdown of phosphatidylinositol 4,5-bis-

Fig. 3. Effect of 2,3-bisphosphoglycerate on inositol phosphate phosphatase activities measured in crude soluble rat brain fraction

Substrates were 5 μM-[³²P]Ins(1,4,5)P₃ (■), 2 μM-[³²P]Ins(1,4)P₂ (●) and 5 μM-[³H]Ins(1)P₁ (▼). Activities are expressed as a percentage of that of enzyme incubated in the absence of 2,3-bisphosphoglycerate (means of triplicates ± S.D.).

phosphate (Berridge & Irvine, 1984). Ins(1,4,5)P₃ is the intracellular messenger of Ca²⁺-mobilizing hormones and neurotransmitters. It is formed by phospholipase C and dephosphorylated by a cascade of soluble and particulate phosphatases. As expected for an important second messenger, the degradation of Ins(1,4,5)P₃, like its synthesis, might be tightly controlled. This is well demonstrated for cyclic AMP and the cyclic AMP phosphodiesterases, which are different enzymes, the activity of which are modified by extracellular (e.g. insulin, thyroid hormones etc.) or intracellular (Ca²⁺, cyclic GMP etc.; Erneux *et al.*, 1985) molecules.

In the present work, Ins(1,4)P₂ dephosphorylation has been measured with [³²P]Ins(1,4)P₂ as substrate. Activities have been estimated from the ³²P eluted in the phosphate peak by Dowex (formate form) chromatography. By this assay, we measure a combined Ins(1,4)P₂ phosphatase activity of two separate pathways yielding [³²P]Ins(4)P₁ and [³²P]P_i (Storey *et al.*, 1984). Ins(1,4)P₂ phosphatase activity, which is predominantly present in the cytosol of rat brain (180 000 g soluble fraction), is different from the Ins(1,4,5)P₃ and Ins(1)P₁ phosphatases of the same fraction. Indeed, those activities can be

Fig. 4. Separation of inositol phosphate phosphatase activities by DEAE-cellulose chromatography of crude supernatant fraction of rat brain homogenate

Experimental details are given in the Materials and methods section. In the first profile (a), substrates were $3 \mu\text{M}$ - $[\text{}^{32}\text{P}]\text{Ins}(1,4,5)\text{P}_3$ (■), $1 \mu\text{M}$ - $[\text{}^{32}\text{P}]\text{Ins}(1,4)\text{P}_2$ (●) and $0.01 \mu\text{M}$ - $[\text{}^3\text{H}]\text{Ins}(1)\text{P}_1$ (▼). In the second profile (b), obtained from a different preparation, substrates were $5 \mu\text{M}$ - $\text{Ins}(1,4)\text{P}_2$ (○, ●) and $0.01 \mu\text{M}$ - $[\text{}^3\text{H}]\text{Ins}(1)\text{P}_1$ (▽, ▼) and the incubation mixture was made in the presence (▽, ○) or absence (▼, ●) of 10 mM - LiCl . Data are means of duplicates and are representative of experiments with three different preparations of rat brain.

separated by DEAE-cellulose chromatography. Although Li^+ inhibits the $\text{Ins}(1)\text{P}_1$ phosphatase, an enzyme well characterized in brain tissue (K_i for Li^+ , 0.8 mM ; Hallcher & Sherman, 1980), our data illustrate that another enzyme dephosphorylates $\text{Ins}(1,4)\text{P}_2$ and is also Li^+ -sensitive. Whether the same mechanism of Li^+ inhibition applies to that $\text{Ins}(1,4)\text{P}_2$ phosphatase activity awaits further enzyme purification and determination of kinetic behaviour.

The observation that 2,3-bisphosphoglycerate, an inhibitor of $\text{Ins}(1,4,5)\text{P}_3$ phosphatase, has no effect on $\text{Ins}(1,4)\text{P}_2$ dephosphorylation, establishes the sharp specificity of the enzymes towards $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,4)\text{P}_2$ as respective substrates. Although these results cannot be considered definitive, they suggest that $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1)\text{P}_1$ phosphatases are different proteins. Moreover, a soluble $\text{Ins}(1,4,5)\text{P}_3$ phosphatase has been purified from human platelets (Connolly *et al.*, 1985). That enzyme is different from that described by Downes *et al.* (1982) in human

erythrocytes, which is membranous and also can hydrolyse glycerophosphoinositol 4,5-bisphosphate. In rat brain, about 20–30% of the $\text{Ins}(1,4,5)\text{P}_3$ phosphatase activity of the homogenate remains in the soluble fraction after high-speed centrifugation (Erneux *et al.*, 1986). This further suggests the existence of a soluble and membrane-associated phosphatase for $\text{Ins}(1,4,5)\text{P}_3$. Conceivably, as has been demonstrated for the cyclic nucleotide phosphodiesterases (Erneux *et al.*, 1985), the activities of any of the phosphatases that use $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1,4)\text{P}_2$ or $\text{Ins}(1)\text{P}_1$ as substrate may vary from one cell type to another.

Li^+ inhibits $\text{Ins}(1,4)\text{P}_2$ dephosphorylation in rat brain. Similar results have been reported by Storey *et al.* (1984) in rat liver. The sensitivity of that enzyme activity to Li^+ may be quite general, which would explain the observation that Li^+ increases agonist-induced $\text{Ins}(1,4)\text{P}_2$ accumulation, reported, for example, in rat hepatocytes (Thomas *et al.*, 1984), pancreatic acini (Rubin, 1984) or human platelets (Siess, 1985). It is

noteworthy that the Li^+ -provoked potentiation of accumulation of inositol phosphates induced by an agonist is reminiscent of the common observation that phosphodiesterase inhibitors have more pronounced effects on cyclic AMP concentrations in stimulated than in resting cells (Erneux *et al.*, 1980).

In conclusion, our data suggest that $\text{Ins}(1)P_1$, $\text{Ins}(1,4)P_2$ and $\text{Ins}(1,4,5)P_3$ phosphatases are different enzymes. Both $\text{Ins}(1,4)P_2$ dephosphorylation and $\text{Ins}(1)P_1$ phosphatase are Li^+ -sensitive. It is now important to compare the profiles of inositol phosphate phosphatases in other cell types or tissues in order to assess whether the products of $\text{Ins}(1,4,5)P_3$ dephosphorylation as seen in intact-cell studies may derive from distinct inositol phosphatases.

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