The interaction of lithium with thyrotropin-releasing hormone-stimulated lipid metabolism in GH₃ pituitary tumour cells

Enhancement of stimulated 1,2-diacylglycerol formation

Alan H. DRUMMOND and Christine A. RAEBURN

Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

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Treatment of GH₃ cells with thyrotropin-releasing hormone (TRH) for periods up to 60 min resulted in a prolonged reduction in the cellular content of phosphatidylinositol (PtdIns) with no lasting change in the levels of the other inositol-containing phospholipids. Accompanying this was a maintained increase in the GH₃ cell 1,2-diacylglycerol content and a slower decline in the level of cellular triacylglycerol. When the cells were suspended in lithium-containing balanced salt solution for 30 min (in the absence of exogenous *myo*-inositol), there was a 15% decrease in GH₃ cell inositol levels. This was associated with a small, but significant, increase in the cellular content of phosphatidylinositol 4,5-bisphosphate (PtdIns4,5P₂) and 1,2-diacylglycerol. Addition of TRH to cells suspended in lithium-containing medium depleted cellular inositol levels by around 65% within 30 min. By this time, there was also a 50% reduction in the cellular content of PtdIns and a 20% reduction in phosphatidylinositol 4-phosphate (PtdIns4P). Control levels of PtdIns4,5P₂ were maintained in the combined presence of TRH and lithium. Under those conditions, TRH no longer depleted cellular triacylglycerol and there was a marked increase in the ability of TRH to elevate the GH₃ cell content of 1,2-diacylglycerol. The effect of TRH on the cellular content of phosphatidic acid was not altered by the presence of lithium. The results show, firstly, that when PtdIns resynthesis is inhibited by lithium-induced inositol depletion, its glycerol backbone accumulates, at least in part, in 1,2-diacylglycerol and, secondly, that GH₃ cells preserve their cellular levels of PtdIns4,5P₂ in the face of a considerable reduction in the cellular content of PtdIns.

For the past three decades, it has been well documented that receptor agonists that mobilize calcium also increase the metabolism of PtdIns (Downes & Michell, 1982). Indeed, Michell has proposed that changes in the metabolism of PtdIns are necessary for calcium mobilization to occur (Michell, 1975). In the past year it has become apparent that the initial step in the alteration of inositol lipid metabolism is the agonist-induced hydrolysis of the polyphosphoinositides, PtdIns4P

and, in particular, PtdIns4,5P₂, by a phospholipase C-type enzyme (for a recent review, see Berridge, 1984). Breakdown of PtdIns4,5P₂ in this fashion yields 1,2-diacylglycerol, which can activate protein kinase C (Takai *et al.*, 1979), and InsP₃, which has recently been shown to mobilize calcium from intracellular stores (Streb *et al.*, 1983). One of the limitations of research in this area of cell biology has been the absence, until recently, of pharmacological agents which perturb the inositol lipid cycle in a specific or even selective manner. Berridge and his collaborators (Berridge *et al.*, 1982) have recently followed up the original investigations of Allison (Allison & Stewart, 1971; Allison & Blisner, 1976) who showed that lithium ions selectively block the enzyme *myo*-inositol 1-phosphomonoesterase and lead, as a consequence, to a large increase in the cellular content of *myo*-inositol 1-

Abbreviations used: TRH, thyrotropin-releasing hormone (thyroliberin); PtdIns, PtdIns4P, PtdIns4,5P₂, phosphatidylinositol and its 4-phosphate and 4,5-bisphosphate; Ins1P, InsP₂, InsP₃, the mono-, bis- and trisphosphates of inositol; PtdA, phosphatidic acid; BSS, balanced salt solution; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

phosphate. Under conditions in which the cell has no source of exogenous *myo*-inositol, the presence of lithium can lead to a relatively rapid reduction of intracellular levels and, subsequently, to a depletion of cellular inositol-containing phospholipids.

GH₃ cells are a clonal line of rat pituitary tumour cells which respond to a number of receptor agonists by releasing prolactin in a calcium-dependent manner (for a review, see Martin & Tashjian, 1977). A number of laboratories, including our own, have recently shown that one of these agents, TRH, also stimulates inositol lipid metabolism in GH₃ cells (Drummond & Macphee, 1981; Rebecchi *et al.*, 1981; Schlegel *et al.*, 1981; Sutton & Martin, 1982). TRH increases the GH₃ cell content of PtdA and 1,2-diacylglycerol and, at the same time, reduces cellular levels of PtdIns by 10–15% (Drummond & Macphee, 1981; Rebecchi *et al.*, 1983; Macphee & Drummond, 1984). Recent work has shown that, in this cell type also, the initial reaction of the cell to the presence of the agonist (TRH) is the hydrolysis of PtdIns4,5P₂ and, perhaps, of PtdIns4P (Rebecchi & Gershengorn, 1983; Martin, 1983; Macphee & Drummond, 1984; Schlegel *et al.*, 1984). GH₃ cells, then, are a convenient model system with which to investigate stimulated inositol lipid metabolism. Previous work from this laboratory has demonstrated that lithium treatment of GH₃ cells can deplete cellular inositol-containing phospholipids (Drummond *et al.*, 1984). However, it remains unclear, in any cell type, whether all three inositol phospholipids are depleted equally under these conditions. The fate of the glycerol backbone of the depleted lipids is also unknown. These are the subjects of the present investigation, which shows, firstly, that GH₃ cells retain their normal levels of PtdIns4,5P₂ despite a large decrease in the cellular PtdIns content and, secondly, that the glycerol backbone of the depleted PtdIns accumulates, at least in part, in 1,2-diacylglycerol.

Experimental

Materials

TRH was obtained from Calbiochem-Behring, C.P. Laboratories, Bishops Stortford, Herts., U.K. Lipid standards and Dowex 1X8 (Cl[−] form, 100–200 mesh) anion-exchange resin were from Sigma. *myo*-[³H]Inositol (16 Ci/mmol) and [2-³H]glycerol (1 Ci/mmol) were from Amersham International. [³²P]Orthophosphate, carrier-free, was from the Western Infirmary, Glasgow, Scotland, U.K. All cell culture reagents and materials were from Flow Laboratories, Irvine, Scotland, U.K. or Gibco-Europe, Paisley, Scotland, U.K. Silica gel G plates were from Camlab, Cambridge, U.K. Silica gel 60 HP-TLC (high-performance t.l.c.) plates (Merck)

were from Macfarlane Robson, Glasgow, Scotland, U.K.

Cell culture

GH₃ cells were grown as previously described (Macphee & Drummond, 1984) in Ham's F10 medium supplemented with antibiotics, horse serum and foetal calf serum. The medium was changed every 3–4 days. Phospholipid studies were generally conducted on GH₃ cells that had been labelled to isotopic equilibrium with an appropriate radiolabelled precursor ([³H]glycerol, [³²P]orthophosphate or [³H]inositol). This was added to the growth medium on the day of the last medium change (usually 4 days before the cells were to be used in an experiment). The data are presented as d.p.m./mg of protein; since the cells have been labelled to equilibrium this is effectively a measure of the cellular content of the metabolite. (The values shown can be converted into molar terms, using the information that the GH₃ cell content of PtdIns is 32.6 nmol/10⁷ cells; see Macphee & Drummond, 1984.) The amount of radioactivity added varied from experiment to experiment and is indicated in the appropriate Figure or Table legend. In one series of experiments, which were designed to measure the rate of incorporation of [³²P]P_i into phospholipids, aliquots of unlabelled cell suspension were incubated with 15 μCi of [³²P]P_i for 30 min. All incubations were carried out on cells suspended in a balanced salt solution (BSS: 135 mM-NaCl, 4.5 mM-KCl, 1.5 mM-CaCl₂, 0.5 mM-MgCl₂, 5.6 mM-glucose, 10 mM-Hepes and 0.1% bovine serum albumin, pH 7.4 at 37°C) (Rebecchi *et al.*, 1981) and prepared as previously described (Macphee & Drummond, 1984). Many of the experiments were carried out with GH₃ cells suspended in BSS containing 10 mM-LiCl. In these cases, the NaCl content of the final suspension had been correspondingly reduced. The protein content of the final suspension was 0.3–0.9 mg/0.5 ml aliquot.

Lipid analyses

Cells in suspension (at 37°C) were split initially into two portions. In one case, the medium in which the cells were suspended was BSS and, in the other, BSS containing 10 mM-LiCl. Each portion was then further subdivided into two test tubes, one of which contained TRH (100 nM) and the other the vehicle (BSS). After incubation for the indicated time, aliquots (0.5 ml) were removed and immediately added to glass test tubes containing 1.5 ml of chloroform/methanol (1:2, v/v). Lipids were then extracted by the acid method of Schacht (1981), dried at 45°C under N₂ and stored at −20°C dissolved in 0.1 ml of chloroform/methanol (9:1, v/v). [³H]Inositol-containing phospholipids

were separated on oxalate-impregnated HP-TLC plates following the procedure of Jolles *et al.* (1981). Neutral lipids were separated on activated silica gel G t.l.c. plates by the method of Calderon *et al.* (1979). All other phospholipids were separated by two-dimensional t.l.c. on silica gel G plates as described by Yavin & Zutra (1977). In all cases, lipids were identified by comparison with reference standards detected with I_2 vapour. Spots/bands were scraped and incorporated radioactivity was quantified by liquid-scintillation spectrometry.

Measurement of cellular inositol and its acid-soluble metabolites

Cell suspensions (0.5 ml), prelabelled with [3H]inositol as indicated in the Table legend, were incubated with or without TRH, in the presence or absence of lithium, for 30 min at 37°C. At the end of the incubation period, 1.5 ml of ice-cold BSS was added to the cells and samples were centrifuged at 650g for 5 min at 4°C. The supernatant, which contains any extracellular inositol, was discarded and the inositol-containing substances present in the cell pellet were released by the addition of 1 ml of 10% (w/v) trichloroacetic acid. These inositol metabolites were measured as previously described (Drummond *et al.*, 1984).

Protein determination

The protein content of the suspended cells was determined by the method of Lowry *et al.* (1951) using bovine serum albumin (Miles) as standard. In each experiment, separate aliquots were taken for this purpose. The cells in these aliquots were washed twice by centrifugation (200g, 3 min) and resuspension in 0.9% (w/v) iso-osmotic saline to remove the 0.1% bovine serum albumin present in BSS. The cell pellet was finally dissolved in 0.1 M NaOH before assay.

Results

Effect of lithium and TRH on the GH_3 cell content of acid-soluble inositol metabolites

The addition of TRH to suspensions of GH_3 cells led to a lasting accumulation of all four inositol-containing metabolites (Drummond *et al.*, 1984, and see Table 1). The presence of 10 mM-lithium in the BSS caused, by itself, a 2.8-fold increase in the cellular $Ins1P$ content within 30 min and also resulted in a small reduction in inositol levels (Table 1). When both TRH and lithium were present there was a very marked potentiation both of $Ins1P$ formation (up to 50-fold above control) and of $InsP_2$ formation (9-fold above control). $InsP_3$ levels were relatively insensitive to the presence of lithium. Under these conditions, greater than 65% of the cellular inositol had been depleted (Table 1).

Effect of lithium and TRH on the incorporation of [^{32}P]P_i into GH_3 phospholipids

As has been shown previously, incorporation of [^{32}P]P_i into GH_3 cell PtdIns and PtdA is markedly stimulated by TRH (Table 2). Under the same circumstances, there is a much smaller stimulation of incorporation into all the other GH_3 cell phospholipids, which is believed to be the result of TRH stimulating [^{32}P]P_i uptake into the acid-soluble fraction of GH_3 cells (Rebecchi *et al.*, 1981; Drummond & Macphee, 1981). When lithium is included in the BSS, there is a reduction, both in the basal and TRH-stimulated states, of [^{32}P]P_i incorporation into PtdIns (Table 2). This occurs without any significant change in the incorporation of isotope into PtdA (Table 2). Note also that the TRH-stimulated incorporation of [^{32}P]P_i into phosphatidylcholine is markedly decreased by the inclusion of lithium.

Table 1. *Effect of lithium and TRH, alone and in combination, on the levels of acid-soluble [3H]inositol metabolites in GH_3 cells*

Cell suspensions (0.5 ml), pre-labelled for 3 days with [3H]inositol (5 μ Ci/dish), were incubated in the presence of the appropriate substances for 30 min at 37°C. The reaction was terminated by the addition of 3 vol. of ice-cold BSS and the metabolites measured as described in the Experimental section. Because of the variation in the absolute levels of the metabolites from experiment to experiment, the results are expressed as percentages of the mean control sample. The average content of each metabolite in control samples (d.p.m./mg of protein) was: [3H]inositol, 21 000 $\pm$ 70; [3H]Ins1P, 3278 $\pm$ 50; [3H]InsP₂, 1220 $\pm$ 200; [3H]InsP₃, 2145 $\pm$ 200. The values shown are the means $\pm$ s.e.m. for four experiments, each conducted in triplicate. These values are significantly different from the control group: * P < 0.05, ** P < 0.01.

Addition	Content (% of mean control sample)			
	[3H]Inositol	[3H]Ins1P	[3H]InsP ₂	[3H]InsP ₃
Control	100.0 $\pm$ 3	100 $\pm$ 4	100 $\pm$ 8	100 $\pm$ 10
LiCl (10mM)	83.5 $\pm$ 5*	282 $\pm$ 57*	121 $\pm$ 13	106 $\pm$ 12
TRH (100nM)	183.6 $\pm$ 13**	388 $\pm$ 54**	204 $\pm$ 28*	188 $\pm$ 16**
LiCl (10mM) $\pm$ TRH (100nM)	34.1 $\pm$ 10**	5115 $\pm$ 1102**	911 $\pm$ 219*	307 $\pm$ 24**

Table 2. *Effect of lithium and TRH, alone and in combination, on the incorporation of [³²P]P_i into a number of GH₃ cell phospholipids*

Cell suspensions (0.5 ml) were incubated in the presence of the appropriate substances for 30 min at 37°C with 15 μ Ci of [³²P]P_i. The reaction was stopped and incorporated radioactivity was determined as described in the Experimental section. The results are expressed as the means $\pm$ S.E.M. for three samples. These values are significantly different from the control group: * P < 0.02, ** P < 0.001.

Addition	Radioactivity incorporated (d.p.m./mg of protein)		
	PtdIns	PtdA	Phosphatidylcholine
Control	50600 $\pm$ 1400	24600 $\pm$ 1000	19200 $\pm$ 320
LiCl (10 mM)	39810 $\pm$ 1860*	27670 $\pm$ 1600	20580 $\pm$ 1240
TRH (100 nM)	793700 $\pm$ 19600**	236700 $\pm$ 8000**	48720 $\pm$ 760**
LiCl (10 mM) + TRH (100 nM)	201400 $\pm$ 6700**	276400 $\pm$ 7000**	26250 $\pm$ 1680*

Table 3. *Effect of lithium and TRH, alone and in combination, on the GH₃ cell content of the inositol-containing phospholipids*

Cell suspensions (0.5 ml), prelabelled for 3 days with [³H]inositol (5 μ Ci/dish), were incubated in the presence of the appropriate substances for 30 min at 37°C. The reaction was stopped and the inositol-containing phospholipids were extracted and estimated as described in the Experimental section. The results are expressed as the means $\pm$ S.E.M. for five samples. These values are significantly different from the control group: * P < 0.05, ** P < 0.02, *** P < 0.001.

Addition	Content (d.p.m./mg of protein)		
	PtdIns	PtdIns4P	PtdIns4,5P ₂
Control	64300 $\pm$ 1200	1820 $\pm$ 45	1310 $\pm$ 60
LiCl (10 mM)	62500 $\pm$ 860	2010 $\pm$ 180	1690 $\pm$ 120*
TRH (100 nM)	55730 $\pm$ 1550**	1700 $\pm$ 60	1400 $\pm$ 120
LiCl (10 mM) + TRH (100 nM)	34200 $\pm$ 500***	1450 $\pm$ 70**	1760 $\pm$ 180

Table 4. *Effect of lithium and TRH, alone and in combination, on the GH₃ cell phospholipid content*

Cell suspensions (0.5 ml), prelabelled for 4 days with [³H]glycerol (100 μ Ci/dish), were incubated in the presence of the appropriate substances for 30 min at 37°C. The reaction was stopped, the phospholipids extracted and their content measured as described in the Experimental section. The results are expressed as the means $\pm$ S.E.M. for five samples. These values are significantly different from the control group: * P < 0.05, ** P < 0.001.

Phospholipid	Addition ...	Content (d.p.m./mg of protein)			
		Control	LiCl (10 mM)	TRH (100 nM)	LiCl (10 mM) + TRH (100 nM)
Phosphatidylinositol		267300 $\pm$ 4700	251900 $\pm$ 4430	210900 $\pm$ 4400**	117200 $\pm$ 5500**
Phosphatidic acid		24260 $\pm$ 1100	24830 $\pm$ 2100	47300 $\pm$ 1300**	47200 $\pm$ 1700**
CDP-diacylglycerol		10320 $\pm$ 800	11600 $\pm$ 940	13900 $\pm$ 750*	18500 $\pm$ 1900*
Phosphatidylethanolamine		435700 $\pm$ 18500	434300 $\pm$ 17500	413900 $\pm$ 15000	449000 $\pm$ 16900
Phosphatidylcholine		604800 $\pm$ 22300	611700 $\pm$ 32000	577600 $\pm$ 11300	540300 $\pm$ 24400
Lysophosphatidylcholine		27600 $\pm$ 3800	32200 $\pm$ 3100	39000 $\pm$ 4400	34950 $\pm$ 4900
Phosphatidylserine		115600 $\pm$ 1500	117300 $\pm$ 2400	88120 $\pm$ 2400**	122300 $\pm$ 5800
Lysophosphatidylethanolamine + sphingomyelin		24420 $\pm$ 1800	27200 $\pm$ 1500	30660 $\pm$ 3400	28500 $\pm$ 2700

Effect of lithium and TRH on the GH₃ cell content of inositol-containing phospholipids

When GH₃ cells were suspended for 30 min in lithium-containing BSS (in the absence of exogenous *myo*-inositol), there was a small, but signifi-

cant, increase in the cellular content of PtdIns4,5P₂ (Table 3). Levels of the other two inositol-containing phospholipids were unaffected by lithium. Treatment of GH₃ cells for 30 min with TRH did not induce a lasting change in their content of PtdIns4,5P₂ or PtdIns4P although there

was a 10–20% reduction in the GH₃ cell PtdIns content (Table 3). When TRH was added to GH₃ cells suspended in lithium-containing medium there was a marked depletion in the PtdIns content (Table 3; 40–60% in a number of experiments). At

the same time, PtdIns4P levels had fallen by 20%, while the PtdIns4,5P₂ content was not significantly different from control values (Table 3).

Effect of lithium and TRH on other GH₃ cell phospholipids

The data presented in Table 4 show that TRH, unlike lithium, also increased the GH₃ cell content of PtdA and CDP-diacylglycerol. Although the effect of TRH on CDP-diacylglycerol levels appeared to be slightly magnified in the presence of lithium, the TRH-induced PtdA accumulation was entirely unaffected (Table 4). The cellular content of a number of other phospholipids was unchanged by the addition of lithium, TRH or a combination of both agents, although the cellular content of phosphatidylserine was reduced by 20–25% when GH₃ cells were incubated with TRH for 30 min (Table 4). This effect was abolished when lithium was present in the BSS along with the peptide.

Effect of lithium and TRH on the neutral lipid content of GH₃ cells

The results presented above indicate that the glycerol backbone of PtdIns, depleted in the presence of both lithium and TRH, does not accumulate as either CDP-diacylglycerol or PtdA and that only a proportion (30%) can potentially be recovered as phosphatidylserine. The most obvious alternative fate of the rest of the PtdIns glycerol backbone is that it is redirected into neutral lipid synthesis. The results shown in Fig. 1 indicate that this is, most likely, the case. Addition of TRH to GH₃ cells elicited a rise in their 1,2-diacylglycerol content which persisted for at least 40 min. In addition, the peptide also induced a fall in the level of triacylglycerol which, while developing more slowly than the effects on 1,2-diacylglycerol, was statistically significant within 40 min and increased over the remainder of the incubation period (Fig. 1). When TRH was added to lithium-containing BSS, there was a marked increase in the ability of TRH to elevate cellular 1,2-diacylglycerol levels, a response that was maintained for at least 1 h (Fig. 1). Interestingly, TRH did not deplete cellular triacylglycerol when it was added to GH₃ cells in the presence of lithium (Fig. 1). Incubation of GH₃ cells in lithium-containing BSS for 60 min resulted, by itself, in a small elevation of 1,2-diacylglycerol levels that was statistically significant within 40 min (Fig. 1).

Discussion

A number of recent studies have emphasized the multi-functional nature of the inositol phospholipid signalling system (Berridge, 1981; Nishizuka, 1984). One of the products of the hydrolysis of

Fig. 1. *Effect of lithium and TRH, alone and in combination, on the GH₃ cell content of 1,2-diacylglycerol and triacylglycerol*

Cell suspensions (0.5 ml), prelabelled for 4 days with [³H]glycerol (100 μ Ci/dish), were incubated with the appropriate substances for times up to 60 min. The reactions were stopped and the neutral lipids extracted and separated as described in the Experimental section. Upper panel: GH₃ cell 1,2-diacylglycerol content as percentage of the mean control sample. Lower panel: GH₃ cell triacylglycerol content. The values shown are the means $\pm$ S.E.M. for nine separate determinations. ▲, control; ●, LiCl (10 mM); △, TRH (100 nM); ○, LiCl (10 mM) + TRH (100 nM). These values are significantly different from the control group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

PtdIns4,5P₂ by phospholipase C, InsP₃, may be involved in short-term changes due to its ability to mobilize cellular calcium (Streb *et al.*, 1983), while the other, 1,2-diacylglycerol, may, by activating protein kinase C, lead to longer-lasting changes in cellular function (Zawilich *et al.*, 1983). It remains unclear whether both of these mechanisms are employed in GH₃ cells, although it has been suggested that the long-term stimulatory effects of TRH on prolactin biosynthesis are related to activation of protein kinase C, while TRH-induced prolactin release may be a response to an increase in cytoplasmic free calcium concentrations (Sobel & Tashjian, 1983). Addition of TRH to suspensions of GH₃ cells leads to a transient increase in the cytoplasmic free calcium concentration (Gershengorn & Thaw, 1983) and to an increase in cellular 1,2-diacylglycerol levels which reaches a steady-state value after 5 min of exposure to the peptide (Rebecchi *et al.*, 1983; Martin, 1983; Macphee & Drummond, 1984). The results presented in the present paper show that the increase in 1,2-diacylglycerol levels, observed after TRH, persists for at least 40–60 min. This is reminiscent of previous work demonstrating long-term changes in PtdA and PtdIns levels after the addition of TRH to GH₃ cells (Macphee & Drummond, 1984), and is consistent with the possibility that the continued activation of protein kinase C by 1,2-diacylglycerol may underlie changes in prolactin synthesis. Previous studies have indicated that the TRH-induced decrease in cellular polyphosphoinositides is a transient phenomenon which has partially (Rebecchi *et al.*, 1983; Martin, 1983) or completely (Macphee & Drummond, 1984) reversed within 5 min. These results agree with the present data which indicate that the levels of PtdIns4,5P₂ are back within the control range 30 min after the addition of TRH.

A primary aim of the present investigation was to determine the fate of the glycerol backbone of the inositol-containing phospholipids as their re-synthesis is inhibited by lithium treatment. The reduction in the cellular concentration of inositol induced by lithium or a combination of lithium and TRH might be expected to compromise re-synthesis of PtdIns, since the K_m of the enzyme CDP-diacylglycerol:myo-inositol transferase for inositol, in liver at least, is 2.5 mM (Takenawa & Egawa, 1977) and the GH₃ cell concentration is calculated to be about 0.3 mM (Drummond *et al.*, 1984). It thus seems likely that the content of inositol will be a limiting factor in GH₃ cell PtdIns biosynthesis. This view receives support from the demonstration that the incorporation of [³²P]P_i into PtdIns, but not into PtdA, is reduced in the presence of lithium.

1,2-Diacylglycerol, the immediate product of

inositol lipid hydrolysis, is situated at an important branch point in neutral and phospholipid biosynthesis, and may potentially be metabolized in a number of ways when its conversion back to PtdIns is inhibited by lithium-induced inositol depletion. The present experiments were conducted in the hope that this pharmacological manipulation would yield information regarding the mechanisms that regulate the intracellular levels of the PtdIns cycle intermediates. The results indicate that lithium treatment causes only a small increase in the TRH-induced elevation of CDP-diacylglycerol and does not enhance TRH-induced PtdA accumulation. The available data indicate, in fact, that the glycerol backbone of the depleted PtdIns accumulates, at least in part, as 1,2-diacylglycerol and may, in addition, be redirected into triacylglycerol and phosphatidylserine biosynthesis. This latter conclusion must be regarded as tentative since it depends on the absence of an effect (the TRH-induced reduction in cellular content of the two lipids) in the presence of both lithium and TRH.

The lack of effect of lithium on TRH-induced PtdA production is interesting, since previous studies from this laboratory have indicated that levels of PtdA increase in response to TRH in GH₃ cells only during the period 10–60 s after the addition of the peptide. This occurs despite a further increase in the availability of its immediate precursor, 1,2-diacylglycerol (Macphee & Drummond, 1984). The obvious conclusion, that factors other than substrate availability control the net activity of the enzymes diglyceride kinase/phosphatidate phosphohydrolase, is borne out by the present findings which indicate that the considerable lithium-induced increase in TRH-elevated 1,2-diacylglycerol levels does not lead to a further increase in the cellular content of PtdA. It remains to be seen what factor(s) controls net phosphatidate production in TRH-stimulated GH₃ cells, but recent work using rat hepatocytes suggests that Ca²⁺-mobilizing agonists may activate phosphatidate phosphohydrolase (Pollard & Brindley, 1984). The present data are entirely consistent with this finding, but further work is obviously necessary to test if TRH can activate the enzyme in GH₃ cells and whether such an effect fully explains the time-course of TRH-induced PtdA production.

Treatment of GH₃ cells with lithium in the absence of an agonist also led to a small increase in their content of 1,2-diacylglycerol. This increase was reliably seen in a number of separate experiments. The source of this 1,2-diacylglycerol remains uncertain, since there was no accompanying change in the cellular levels of any other lipid. It should be noted, however, that the amount of 1,2-

diacylglycerol formed was relatively small and, had it been produced from PtdIns, for example, the resultant decline in the PtdIns content would have been around 3–5%. Such a change would have been very difficult to detect. The most likely source of the 1,2-diacylglycerol is probably PtdIns (presumably via polyphosphoinositide hydrolysis), since lithium does lead to a small decline in the cellular content of inositol under those conditions. Whatever the mechanism, the findings are of some interest since there is no accompanying change in the cellular content of InsP₃ when cells are treated with lithium. If, as Berridge and his collaborators suggest (Streb *et al.*, 1983), this inositol metabolite is involved in the mobilization of cellular calcium, treatment of cells and tissues with lithium may, under certain conditions, lead to selective activation of protein kinase C in the absence of a change in cytoplasmic free calcium levels. This pharmacological manipulation may, thus, produce similar effects to the addition of phorbol esters which, as Castagna *et al.* (1982) have recently shown, directly activate protein kinase C. The enhanced accumulation of 1,2-diacylglycerol observed with TRH under inositol-depleting conditions might have a similar significance, in terms of protein kinase C activation, for certain pharmacological and pathological circumstances such as the treatment of mania with lithium and in diabetic neuropathy, both of which are thought to be associated with a decreased cellular inositol content (Allison & Stewart, 1971; Gillon & Hawthorne, 1983).

The second primary reason for conducting these studies was to investigate whether all three inositol-containing phospholipids are equally sensitive to depletion by lithium. This does not appear to be the case, since GH₃ cells maintain normal levels of PtdIns4,5P₂ in the face of a considerable loss of the precursor PtdIns: an indicator, perhaps, of the importance to the cell of preserving high PtdIns4,5P₂ levels. The cellular content of PtdIns4P is decreased by 20% under the same conditions. A number of laboratories have suggested that the agonist, in this case TRH, must stimulate both PtdIns4,5P₂ hydrolysis and resynthesis during activation of the inositol lipid cycle (Downes & Wusteman, 1983; Litosch *et al.*, 1983; Drummond *et al.*, 1984). It is difficult, in the absence of such a dual activation, to explain how PtdIns4,5P₂ levels return relatively rapidly to the control range when the cycle is clearly still in an activated state. The data presented here reinforce this notion, since polyphosphoinositide levels remain high even when the cellular content of their immediate precursor is markedly decreased by treatment of the cells with TRH in the presence of lithium. The mechanisms involved in stimulating PtdIns4,5P₂ resynthesis remain unknown, although it should be

noted that there is some evidence that the enzymes of polyphosphoinositide synthesis, in particular PtdIns4P kinase, are subject to control by phosphorylation (Jolles *et al.*, 1980, 1981; Enyedi *et al.*, 1983; Akhtar *et al.*, 1983). Gispén and his colleagues (Aloyo *et al.*, 1982) have shown that a kinase which is similar to, if not identical with, protein kinase C controls PtdIns4P kinase in rat brain. Further work is obviously necessary to resolve the mechanism underlying agonist-stimulated PtdIns4,5P₂ resynthesis.

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