Reduced inositol polyphosphate accumulation and inositol supply induced by lithium in stimulated cerebral cortex slices

Eleanor D. KENNEDY,* R. A. John CHALLISS,* C. Ian RAGAN† and Stefan R. NAHORSKI*‡

*Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, Leicester LE1 9HN, and †Merck, Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex CM20 2QR, U.K.

The ability of lithium to interfere with phosphoinositide metabolism in rat cerebral cortex slices has been examined by monitoring the accumulation of CMP-phosphatidate (CMP-PtdOH) and the reduction in $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ levels. A small accumulation of [^{14}C]CMP-PtdOH was seen in slices prelabelled with [^{14}C]cytidine and stimulated with carbachol (1 mM) or Li^+ (1 mM). However, simultaneous addition of both agents for 30 min produced a 22-fold accumulation, with Li^+ producing a half-maximal effect at a concentration of 0.61 ± 0.19 mM. Kinetic studies revealed that the effects of carbachol and Li^+ on CMP-PtdOH accumulation occurred with no initial lag apparent under these conditions and that preincubation with *myo*-inositol (10 or 30 mM) dramatically attenuated CMP-PtdOH accumulation. *myo*-Inositol could also attenuate the rate of accumulation of CMP-PtdOH when added 20 min after carbachol and Li^+ ; these effects were not observed when equimolar concentrations of *scyllo*-inositol were added. Use of specific radioreceptor assays allowed the mass accumulations of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ to be monitored. Following a lag of 5–10 min, Li^+ resulted in a marked reduction in the accumulation of both inositol polyphosphates resulting from muscarinic-cholinergic stimulation. Preincubation of cerebral cortex slices with *myo*- (but not *scyllo*-) inositol delayed, but did not prevent, the reduction in the accumulation of $\text{Ins}(1,4,5)\text{P}_3$ or $\text{Ins}(1,3,4,5)\text{P}_4$. The results suggest that cerebral cortex, at least *in vitro*, is very sensitive to *myo*-inositol depletion under conditions of muscarinic receptor stimulation. The relationship of such depletion to the generation of inositol polyphosphate second messengers is discussed.

INTRODUCTION

In the last few years there has been growing evidence that the action of Li^+ in affective disorders is related to its ability to interfere with phosphoinositide signalling (Berridge *et al.*, 1982; Drummond, 1987; Sherman, 1989; Berridge *et al.*, 1989). Li^+ is a potent uncompetitive inhibitor of inositol monophosphatase (Hallcher & Sherman, 1980; Gee *et al.*, 1988) and several studies have shown large accumulations of inositol monophosphates and a more modest (though stoichiometrically similar) reduction in inositol in agonist-stimulated Li^+ -treated cerebral tissue (Allison & Stewart, 1971; Berridge *et al.*, 1982; Batty & Nahorski, 1985, 1987). It has been argued that Li^+ might act by reducing the supply of inositol for the resynthesis of phosphoinositides that are used to generate inositol polyphosphate second messengers. Although it has not been easy to provide strong evidence for such a model [indeed, in some cell types, $\text{PtdIns}(4,5)\text{P}_2$ appears resistant to depletion by Li^+ (Drummond & Raeburn, 1984; Downes & Stone, 1986)], a number of studies on cerebral slices have revealed potent inhibitory effects of this ion on muscarinic-agonist-stimulated $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ accumulation (Batty & Nahorski, 1985, 1987; Whitworth & Kendall, 1988; Kennedy *et al.*, 1989).

In an attempt to provide more direct evidence for this hypothesis, we have examined the accumulation of CMP-phosphatidate (CMP-PtdOH) in rat cerebral cortex following muscarinic agonist stimulation in the presence of Li^+ . CMP-PtdOH is an immediate precursor of the phosphoinositides and in view of the relatively high K_m (2.5 mM) for inositol of the enzyme CMP-PtdOH:*myo*-inositol transferase (CDP-diacylglycerol:inositol 3-phosphatidyltransferase; EC 2.7.8.11) (Takewawa & Egawa, 1977), accumulation of this metabolite would

be expected to be a sensitive indicator of inositol depletion. CMP-PtdOH was first shown to accumulate in pancreatic islets stimulated with glucose (Freinkel *et al.*, 1975) and, more recently, enormous amplification of CMP-PtdOH accumulation by Li^+ has been reported in agonist-stimulated parotid gland (Downes & Stone, 1986) and cerebral cortex (Godfrey, 1989). Here we use this approach, in parallel with mass measurements of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$, to examine this potential mechanism of the action of Li^+ in brain, and examine the ability of exogenously added *myo*- and *scyllo*-inositol to reverse the observed effects.

MATERIALS AND METHODS

Materials

[U- ^{14}C]Cytidine (0.5 Ci/mmol) was purchased from Amersham International (Amersham, Bucks., U.K.). [^3H]Ins(1,4,5) P_3 (17–20 Ci/mmol) and [^{32}P]Ins(1,3,4,5) P_4 (250 Ci/mmol) were generously provided by NEN DuPont (U.K.) Ltd. (Stevenage, Herts., U.K.). *myo*-Inositol was purchased from Sigma and *scyllo*-inositol was synthesized at the Merck, Sharp and Dohme Research Laboratories. All other reagents were of analytical grade.

Incubation techniques

Cerebral cortex from male Wistar rats (200–250 g) was cross-chopped ($350 \mu\text{m} \times 350 \mu\text{m}$) and incubated in flat-bottomed flasks for 60 min at 37 °C in 100 ml of Krebs–Henseleit bicarbonate (KHB) buffer [in mM: NaCl, 118; KCl, 4.7; CaCl_2 , 1.3; KH_2PO_4 , 1.2; NaHCO_3 , 25; glucose, 10, pH 7.4, saturated with O_2/CO_2 (19:1)], with replacement of buffer at 15 min intervals. Gravity-packed slices (50 μl), in a final volume of 300 μl ,

Abbreviations used: PtdOH, phosphatidate; KHB buffer, Krebs–Henseleit bicarbonate buffer; TRH, thyrotropin-releasing hormone.

‡ To whom correspondence should be addressed.

were incubated in the absence or presence of [^{14}C]cytidine (0.1 $\mu\text{Ci}/\text{vial}$) for a further 60 min.

All additions of carbachol (1 mM) and LiCl were made in a volume of 10 μl . Incubations were terminated by addition of 300 μl of ice-cold 1 M-trichloroacetic acid, allowed to extract for 15–20 min on ice and then centrifuged (3000 g, 20 min, 4 $^{\circ}\text{C}$).

[^{14}C]CMP-PtdOH determination

For [^{14}C]cytidine-labelled samples, supernatants were removed and 1 ml of 5% (w/v) trichloroacetic acid containing 1 mM-EDTA was added to the pellets. Slices were agitated gently and centrifuged (as above). The supernatants were aspirated, slices were washed in distilled water and recentrifuged, and the supernatants were again aspirated and discarded. Portions of acidified methanol/chloroform [0.94 ml; 2:1 (v/v) containing 0.1 M-HCl] were added and the samples were extracted for 10–15 min. Phases were separated by addition of 0.31 ml of chloroform and 0.56 ml of 0.1 M-HCl and centrifuged (as above). A sample of the lower phase (0.4 ml) was removed and evaporated to dryness, and radioactivity was determined by liquid scintillation counting.

Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 assay

Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 concentrations were determined in tissue extracts after removal of trichloroacetic acid by washing with 4 $\times$ 3 vol. of water-saturated diethyl ether and addition of NaHCO_3 and EDTA (final concns. 10 mM and 5 mM respectively). Ins(1,4,5) P_3 concentration was determined using a radioreceptor assay (Challiss *et al.*, 1988). Ins(1,3,4,5) P_4 concentration was measured using a modification of the method described by Donié & Reiser (1989). Samples of extract (50 μl) were added to a 100 μl of buffer containing 50 mM-sodium acetate, 50 mM- KH_2PO_4 and 2 mM-EDTA, pH 5.0, containing 2 mg of bovine serum albumin/ml and [^{32}P]Ins(1,3,4,5) P_4 (20000 d.p.m.; 0.18 nM in assay). Assays were started by addition of 0.3 mg of protein of a 'P₂' membrane preparation (50 μl) prepared from rat cerebellum. After 30 min at 4 $^{\circ}\text{C}$, bound and free species were separated by rapid filtration over GF/B filters and washed with 3 $\times$ 3 ml of 25 mM-sodium acetate/25 mM- KH_2PO_4 /5 mM- NaHCO_3 /1 mM-EDTA, pH 5.0, at 4 $^{\circ}\text{C}$.

For both assays, standard amounts of Ins(1,4,5) P_3 or Ins(1,3,4,5) P_4 were diluted in a similarly prepared buffer extract (i.e. ether-extracted KHB/trichloroacetic acid containing NaHCO_3 and EDTA) to give Ins(1,4,5) P_3 concentrations of 1–300 nM and Ins(1,3,4,5) P_4 concentrations of 0.3–1000 nM. For the Ins(1,3,4,5) P_4 assay, total binding under the conditions described, in the absence of competing Ins(1,3,4,5) P_4 , was about 1200 d.p.m. with the non-specific component [defined in the presence of 50 μM -DL-Ins(1,3,4,5) P_4] being less than 15% of total binding. Preliminary experiments confirmed the remarkable specificity of this radioreceptor assay for the Ins(1,3,4,5) P_4 isomer reported previously (Donié & Reiser, 1989), with Ins(1,3,4,5,6) P_5 , Ins(1,4,5,6) P_4 and Ins(1,3,4,6) P_4 all showing more than 100-fold lower affinity for the rat cerebellar binding site under the assay conditions described (Challiss & Nahorski, 1990).

RESULTS

In preliminary experiments, various periods of preincubation with [^{14}C]cytidine were examined to achieve optimal labelling of [^{14}C]CMP-PtdOH. Preincubation with [^{14}C]cytidine for either 30 or 60 min resulted in virtually identical accumulations of [^{14}C]CMP-PtdOH when cerebral cortex slices were incubated in the presence of 1 mM-carbachol and 5 mM- Li^+ for a further 30 min (results not shown). In view of the proposed kinetic

Fig. 1. Concentration-dependent effect of Li^+ on the accumulation of [^{14}C]CMP-PtdOH in cerebral cortex slices

Cerebral cortex slices were incubated in the presence of Li^+ (○) or Li^+ plus 1 mM-carbachol (●) for 30 min. Results are presented as means $\pm$ S.E.M. for six experiments, each performed in triplicate. The concentration of Li^+ causing half-maximal stimulation of CMP-PtdOH accumulation was determined (DeLean *et al.*, 1978) to be 0.61 ± 0.19 mM. Addition of 1 mM-carbachol in the absence of Li^+ for 30 min increased CMP-PtdOH accumulation by 58% [basal, 229 ± 11 ; 1 mM-carbachol, 362 ± 16 d.p.m./0.4 ml (means $\pm$ S.E.M. for four experiments)].

studies, we routinely adopted a 60 min period of preincubation with [^{14}C]cytidine.

Radioactivity appearing in the organic phase following methanol/chloroform extraction was assumed to be CMP-PtdOH on the basis that it is the only known lipid that contains cytidine, and other studies on rat cerebral cortex slices have shown that under identical conditions more than 90% of the radioactivity runs identically to CMP-PtdOH on three separate solvent t.l.c. systems (Godfrey, 1989).

Other experiments revealed that changes in the ionic strength of the extracellular medium caused by the addition of various concentrations of Li^+ (up to 30 mM) did not influence the production of [^{14}C]CMP-PtdOH *per se*. However, Li^+ greatly enhanced the accumulation of [^{14}C]CMP-PtdOH when slices were incubated with carbachol for 30 min (Fig. 1). In the absence of Li^+ , stimulation of cerebral cortex slices with carbachol resulted in a small accumulation ($58 \pm 8\%$ increase over basal accumulation) of this metabolite. However, a concentration-dependent enhancement was observed when Li^+ was also present (Fig. 1), with the concentration of Li^+ causing a half-maximal increase in CMP-PtdOH accumulation in carbachol-stimulated cerebral cortex slices being 0.61 ± 0.19 mM (mean $\pm$ S.E.M. for six experiments).

In the presence of 1 mM- Li^+ , the accumulation of [^{14}C]CMP-PtdOH was linear with time for at least 20 min, with no lag-phase apparent (Fig. 2). In agreement with Godfrey (1989), the effects of Li^+ on [^{14}C]CMP-PtdOH accumulation in carbachol-stimulated slices could be dramatically reduced when the slices were preincubated with *myo*-inositol (10 or 30 mM) for 60 min (Fig. 2). The effectiveness of *myo*-inositol was also clearly revealed when it was added to slices subsequent to agonist and Li^+

Fig. 2. Effect of *myo*-inositol on the time-courses of accumulation of $[^{14}\text{C}]\text{CMP-PtdOH}$ in the presence of Li^+ and carbachol in cerebral cortex slices

Li^+ (1 mM) and carbachol (1 mM) were added at t_0 , after preincubation of the slices for 60 min in the absence (●) or presence of 10 mM- (■) or 30 mM- (▲) *myo*-inositol. Results are presented as means $\pm$ S.E.M. for at least three experiments, each performed in triplicate. The presence of *myo*-inositol (10 or 30 mM) had no effect on basal CMP-PtdOH accumulation at t_0 , but significantly attenuated the increase in accumulation subsequent to Li^+ and carbachol addition ($P < 0.01$ for all data points, Student's t test).

Fig. 3. Effects of subsequent addition of *myo*-inositol or *scyllo*-inositol on the accumulation of $[^{14}\text{C}]\text{CMP-PtdOH}$ in the presence of Li^+ and carbachol in cerebral cortex slices

Li^+ (1 mM) and carbachol (1 mM) were added at t_0 (●). After 20 min, final concentrations of 10 mM (■) or 30 mM- (▲) *myo*-inositol or 10 mM-*scyllo*-inositol (○) were added. Results are presented as means $\pm$ S.E.M. for at least three experiments, each performed in triplicate. The addition of *myo*-inositol (10 or 30 mM) significantly attenuated the increase in accumulation of CMP-PtdOH ($P < 0.05$ for data-points, Student's t -test); *scyllo*-inositol addition did not significantly affect CMP-PtdOH accumulation.

addition (Fig. 3). An immediate inhibition of the rate of $[^{14}\text{C}]\text{CMP-PtdOH}$ accumulation was observed with 10 mM-*myo*-inositol, and an almost complete suppression was apparent after addition of 30 mM-*myo*-inositol. In contrast, preincubation with, or subsequent addition (Fig. 3) of 10 mM-*scyllo*-inositol [a naturally occurring stereoisomer of *myo*-inositol, which is a substrate for the *myo*-inositol transport system in cerebral cortex (Spector, 1976)] was totally ineffective in reversing the action of Li^+ on $[^{14}\text{C}]\text{CMP-PtdOH}$.

We have also examined, in parallel experiments, the ability of Li^+ to reduce the accumulation of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ following carbachol stimulation of cerebral cortex slices (Fig. 4). In the absence of Li^+ , carbachol resulted in a rapid and maintained increase in $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ concentrations. Muscarinic-agonist stimulation resulted in a 40–50% increase in the accumulation of $\text{Ins}(1,4,5)\text{P}_3$ and a pronounced 30–40-fold increase in the $\text{Ins}(1,3,4,5)\text{P}_4$ concentration, with the elevated levels of both inositol polyphosphate second messengers being maintained for at least 20 min.

It should be noted that the specificity of these assays is substantial, with generally less than 1% cross-reactivity with other isomers. Perhaps the most likely source of error would be due to the presence of $\text{Ins}(1,3,4,5,6)\text{P}_5$, which may be present in tissues in high concentrations. However, the cross-reactivity of this compound in the $\text{Ins}(1,3,4,5)\text{P}_4$ assay is only about 0.25%.

and it is therefore unlikely to significantly influence the results in cerebral cortex slices reported here.

In agreement with previous experiments (Kennedy *et al.*, 1989), 1 mM- Li^+ reduced the mass accumulation of $\text{Ins}(1,4,5)\text{P}_3$ after a lag of 5–10 min (Fig. 4a). This inhibitory effect of Li^+ was also seen on the mass accumulation of $\text{Ins}(1,3,4,5)\text{P}_4$ (Fig. 4b).

In an attempt to determine whether the decreased agonist-induced accumulation of inositol polyphosphates is related to the ability of Li^+ to inhibit inositol monophosphatase and remove an important source of inositol for phosphoinositide synthesis, slices were preincubated with 10 mM-*myo*-inositol. Under these conditions, a partial reversal of the inhibitory effects of 1 mM- Li^+ was observed. Preincubation of slices with 10 mM-*myo*-inositol did not alter significantly the carbachol-stimulated responses, but the inhibitory effect of Li^+ (1 mM) was delayed, though not totally suppressed. This is particularly evident on comparison of the responses at 10 min: in carbachol- and Li^+ -treated slices, the presence of 10 mM-*myo*-inositol resulted in the concentrations of both $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ being maintained at elevated levels, whereas the concentrations of both second messengers decreased significantly in the absence of added *myo*-inositol (Fig. 4).

The protective effect of *myo*-inositol was not significantly improved by raising its concentration to 30 mM. *scyllo*-Inositol

Fig. 4. Effects of Li^+ and *myo*-inositol on the time courses of changes in $\text{Ins}(1,4,5)\text{P}_3$ (a) and $\text{Ins}(1,3,4,5)\text{P}_4$ (b) concentrations in carbachol-stimulated cerebral cortex slices

Carbachol (1 mM) only ($\circ$, $\bullet$), or carbachol plus 1 mM- Li^+ ($\square$, $\blacksquare$) were added at t_0 , after preincubation of slices in the absence ($\circ$, $\square$) or presence ($\bullet$, $\blacksquare$) of 10 mM-*myo*-inositol for 60 min. Results are presented as means $\pm$ S.E.M. for at least three experiments, each performed in triplicate. Statistically significant attenuating effects of inositol on carbachol-stimulated inositol polyphosphate accumulation by Li^+ are indicated as * P < 0.05; ** P < 0.01; *** P < 0.001.

(10 mM) was completely ineffective in preventing the inhibitory effect of Li^+ on inositol polyphosphate accumulation (results not shown).

DISCUSSION

The ability of cells to maintain receptor-mediated phosphoinositide metabolism depends upon lipid resynthesis and a supply of inositol. Although cells can take up this cyclitol from the extracellular medium, it would appear, from the present study on cerebral cortex slices *in vitro*, that this is not a particularly efficient process. There is increasing evidence that Li^+ may exert its relatively subtle effects in the central nervous system, particularly in relation to its effectiveness in manic-depressive illness, by reducing the supply of inositol for phosphoinositide synthesis (Berridge *et al.*, 1982, 1989; Drummond, 1987). In particular, the uncompetitive nature of the inhibition exerted by Li^+ on inositol monophosphatase provides a strong stimulus-dependency on this agent, with those cells undergoing marked agonist stimulation being selectively deprived of a potentially crucial source of inositol.

The results from this study clearly reveal that there are rapid and very sensitive effects of Li^+ on phosphoinositide synthesis, such that significant accumulation of CMP-PtdOH occurs without an apparent lag and at concentrations of Li^+ that, in cerebral cortex, are less than half-maximally effective in inhibiting inositol monophosphatase activity. This evidence suggests strongly that cerebral tissue (at least *in vitro*) is exquisitely sensitive to modest depletion of inositol, and that in agonist-stimulated tissue a major source of the cyclitol derives from its recovery from inositol polyphosphate dephosphorylation. In this respect, our data support those reported recently by Godfrey (1989) using [^{14}C]cytidine labelling of CMP-PtdOH in rat cerebral cortex

slices, and those of Downes & Stone (1986) in parotid gland. It should be noted, however, that in the latter tissue a lag of 15 min occurred before Li^+ caused an elevation of CMP-PtdOH concentration, suggesting that in parotid gland there may be a larger or more accessible additional source of inositol than in brain slices. The lack of any marked accumulation of CMP-PtdOH in slices stimulated in the absence of Li^+ suggests that in brain the recycling pathway provides, and efficiently conserves, sufficient inositol to maintain phosphatidylinositol resynthesis. However, in the presence of even submillimolar Li^+ , lipid synthesis becomes impaired, as clearly indicated by the accumulation of CMP-PtdOH (Fig. 2).

Despite the overwhelming evidence that Li^+ should severely reduce inositol lipid synthesis in stimulated cells, this has proved difficult to establish using labelling techniques. Thus in parotid gland (Downes & Stone, 1986) and in GH_3 cells (Drummond & Raeburn, 1984) labelling of $\text{PtdIns}(4,5)\text{P}_2$ was relatively unaffected by Li^+ . In carbachol-stimulated cerebral cortex slices, Li^+ also fails to affect the labelling of this lipid by more than 15% (I. H. Batty & S. R. Nahorski, unpublished work). However, it is not clear what proportion of these labelled lipids represents agonist-sensitive pools even within single cells (Monaco & Woods, 1983); in brain slices, which contain heterogeneous cell populations, such changes in agonist-sensitive phosphoinositide pools would be expected to be totally masked. This highlights the enormous advantage of using CMP-PtdOH accumulation, since this will select the agonist-sensitive impairment of lipid synthesis. As argued above, it is the most actively stimulated cells that will be particularly sensitive to Li^+ , and this may provide the basis for some aspects of the cell specificity for Li^+ (Berridge *et al.*, 1989) and the apparent agonist specificity of Li^+ action on $\text{Ins}(1,3,4,5)\text{P}_4$ accumulation reported by Whitworth & Kendall (1988) in brain slices. Thus less effective agonists (by virtue of receptor density

or coupling to phosphoinositidase C) lead to less Li^+ impairment of lipid synthesis, as assessed by CMP-PtdOH accumulation (Godfrey, 1989).

It seems certain that the ability of Li^+ to suppress phosphoinositide synthesis in cerebral cortex slices closely relates to the effect of Li^+ on carbachol-stimulated accumulation of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$. In previous studies (Batty & Nahorski, 1985, 1987; Kennedy *et al.*, 1989; Rooney & Nahorski, 1989) and the present study, Li^+ produced a marked reduction in the polyphosphates after a lag of 5–10 min. The effect is concentration-dependent, with remarkably similar dose-response curves to those seen for inositol monophosphate and CMP-PtdOH accumulation (Kennedy *et al.*, 1989; Godfrey, 1989; present results). These data strongly suggest that inositol depletion leads to a reduced ability of cells to sustain the generation/accumulation of inositol polyphosphate second messengers. Recent studies by Balla *et al.* (1988) in adrenal glomerulosa cells have also revealed major inhibitory effects of Li^+ on angiotensin-induced accumulation of $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$ and $[\text{H}]\text{Ins}(1,3,4,5)\text{P}_4$. However, no such effects of Li^+ were seen in thyrotropin-releasing-hormone (TRH)-stimulated GH_3 cells, despite the ability of these cells to sustain an accumulation of these polyphosphates (Hughes & Drummond, 1987). These apparently contradictory reports may again highlight differences in inositol supply within cells, the size of the hormone-sensitive $\text{PtdIns}(4,5)\text{P}_2$ pool and the strength of stimulus at phosphoinositidase C. Of considerable significance in this respect is evidence that Li^+ and TRH result in only a very small accumulation of labelled CMP-PtdOH in GH_3 cells (Drummond & Raeburn, 1984) implying a minor Li^+ impairment of agonist-sensitive pools of lipids in the cells.

Further support for the inositol-depletion model of Li^+ action is provided in the present studies with experiments in which slices are preincubated with *myo*- and *scyllo*-inositol. Development of assays to measure the mass of $\text{Ins}(1,4,5)\text{P}_3$ (Challiss *et al.*, 1988) and $\text{Ins}(1,3,4,5)\text{P}_4$ (Donié & Reiser, 1989) has allowed us to test the ability of *myo*-inositol to prevent the accumulation of CMP-PtdOH and the loss of the polyphosphates without the complications of altering the specific activity of the $[\text{H}]\text{inositol}$ -labelled species normally studied. These experiments have revealed clear effects of *myo*-inositol, but not of its isomer *scyllo*-inositol (which is recognized by the inositol transporter system but is not a substrate for CMP-PtdOH:*myo*-inositol transferase), to reduce the effects of Li^+ on CMP-PtdOH accumulation and to increase the lag before Li^+ reduces $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ accumulation. These data have also established how difficult it is to overcome the effects of Li^+ in brain slices, since 10 and 30 mM-*myo*-inositol do not totally overcome the effects on CMP-PtdOH, and only delay the effects on the inositol polyphosphates. This could, in part, relate to difficulties in loading cerebral cortex slices in which substantial losses of inositol occur during preparation (Sherman *et al.*, 1986). However, it may also indicate poor uptake and highlight the tight coupling of phosphoinositide hydrolysis and inositol recovery by dephosphorylation of inositol phosphates in stimulated cerebral cortex. This again emphasizes that even slight impairment of inositol lipid synthesis can lead to reduced inositol polyphosphate accumulation.

Despite this overwhelming evidence that *myo*-inositol supply is crucial to phosphoinositide signalling, we still have concerns about a simple model of impairment of $\text{PtdIns}(4,5)\text{P}_2$ synthesis (Batty & Nahorski, 1987; Kennedy *et al.*, 1989). If the assumption is made that the vast majority of InsP_1 arises from dephosphorylation of inositol polyphosphates under these conditions (see Batty & Nahorski, 1989; Batty *et al.*, 1989), then it is difficult to reconcile a linear accumulation of the monophosphates under conditions where the concentrations of inositol polyphosphates are markedly decreased. We have previously

discussed an indirect action of Li^+ (possibly via protein kinase C) on the 5-phosphatase step, such that a smaller but more rapidly turning-over pool of inositol polyphosphates exists under these conditions (Kennedy *et al.*, 1989). Other possibilities could include shifts in the lipid source of phosphoinositidase C (i.e. PtdIns or $\text{PtdIns}4\text{P}$) or a greater involvement of cyclic (poly)-phosphates in the presence of Li^+ . These possibilities require substantial further investigation.

In conclusion however, we feel that the present data provide substantial evidence for the hypothesis that Li^+ can impair phosphoinositide synthesis and that this (directly or indirectly) results in a reduced accumulation of inositol polyphosphate second messengers. It would be of considerable interest to know whether clear functional effects of Li^+ in brain (e.g. Worley *et al.*, 1988) can be inhibited by *myo*-inositol, as there is growing evidence for such reversals in the action of Li^+ in various cell division and embryonic development models (Forer & Sillers, 1987; Busa & Gimlich, 1989), as well as in lymphocyte activation (Mustelin *et al.*, 1986) and in α_1 -adrenoceptor effects on the heart (Mantelli *et al.*, 1988). Collectively, this strongly implicates phosphoinositide metabolism in multiple cellular functions and highlights the potent and selective actions of Li^+ on this signalling system.

We thank the Wellcome Trust for financial support. E.D.K. is supported by a SERC/CASE research studentship with Merck, Sharp and Dohme. We thank Mrs. Lyn McCarthy for manuscript preparation.

REFERENCES

- Allison, J. H. & Stewart, M. A. (1971) *Nature* (London) **233**, 267–268
- Balla, T., Baukal, A. J., Guillemette, G. & Catt, K. J. (1988) *J. Biol. Chem.* **263**, 4083–4091
- Batty, I. H. & Nahorski, S. R. (1985) *J. Neurochem.* **45**, 1514–1521
- Batty, I. H. & Nahorski, S. R. (1987) *Biochem. J.* **247**, 707–800
- Batty, I. H. & Nahorski, S. R. (1989) *Biochem. J.* **260**, 237–241
- Batty, I. H., Letcher, A. & Nahorski, S. R. (1989) *Biochem. J.* **258**, 23–32
- Berridge, M. J., Downes, C. P. & Hanley, M. R. (1982) *Biochem. J.* **206**, 587–595
- Berridge, M. J., Downes, C. P. & Hanley, M. R. (1989) *Cell* **59**, 411–419
- Busa, W. B. & Gimlich, R. L. (1989) *Dev. Biol.* **132**, 315–324
- Challiss, R. A. J. & Nahorski, S. R. (1990) *J. Neurochem.* **54**, in the press
- Challiss, R. A. J., Batty, I. H. & Nahorski, S. R. (1988) *Biochem. Biophys. Res. Commun.* **157**, 684–691
- DeLean, A., Munson, P. J. & Rodbard, D. (1978) *Am. J. Physiol.* **235**, E97–E102
- Donié, F. & Reiser, G. (1989) *FEBS Lett.* **254**, 155–158
- Downes, C. P. & Stone, M. A. (1986) *Biochem. J.* **234**, 199–204
- Drummond, A. H. (1987) *Trends Pharmacol. Sci.* **8**, 129–133
- Drummond, A. H. & Raeburn, C. A. (1984) *Biochem. J.* **224**, 129–136
- Forer, A. & Sillers, P. J. (1987) *Exp. Cell Res.* **170**, 42–55
- Freinkel, N., El Younsi, C. & Dawson, R. M. C. (1975) *Eur. J. Biochem.* **59**, 245–252
- Gee, N. S., Ragan, C. I., Wating, K. J., Aspley, S., Jackson, R. G., Reid, G. G., Gani, D. & Shute, J. K. (1988) *Biochem. J.* **249**, 883–889
- Godfrey, P. P. (1989) *Biochem. J.* **258**, 621–624
- Hallcher, L. M. & Sherman, W. R. (1980) *J. Biol. Chem.* **255**, 10896–10901
- Hughes, P. J. & Drummond, A. H. (1987) *Biochem. J.* **248**, 463–470
- Kennedy, E. D., Challiss, R. A. J. & Nahorski, S. R. (1989) *J. Neurochem.* **53**, 1652–1655
- Mantelli, L., Capanni, L., Corti, V., Bennardini, F., Matucci, R. & Ledda, F. (1988) *Eur. J. Pharmacol.* **150**, 123–129
- Monaco, M. E. & Woods, D. (1983) *J. Biol. Chem.* **258**, 15125–15129
- Mustelin, T., Pösö, H., Iivanainen, A. & Andersson, L. C. (1986) *Eur. J. Immunol.* **16**, 859–861
- Rooney, T. A. & Nahorski, S. R. (1989) *Eur. J. Pharmacol.* **172**, 425–434

- Sherman, W. H. (1989) in *Inositol Lipids in Cell Signalling* (Michell, R. H., Drummond, A. H. & Downes, C. P., eds.), pp. 39–79, Academic Press, London
- Sherman, W. R., Gish, B. G., Honchar, M. P. & Munsell, L. Y. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 2639–2646
- Spector, R. (1976) *J. Neurochem.* **27**, 1273–1276
- Takenawa, T. & Egawa, K. (1977) *J. Biol. Chem.* **252**, 5419–5423
- Whitworth, P. & Kendall, D. A. (1988) *J. Neurochem.* **51**, 258–265
- Worley, P. F., Heller, W. A., Snyder, S. H. & Baraban, J. M. (1988) *Science* **239**, 1428–1429

Received 27 November 1989/23 January 1990; accepted 30 January 1990