Lithium-induced reduction in intracellular inositol supply in cholinergically stimulated parotid gland

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The effects of lithium and cholinergic stimulation on inositol phospholipid metabolism have been assessed using rat parotid gland slices and isolated acinar cells labelled with $^{32}\text{P}_i$. Cholinergic stimulation using carbachol caused substantial breakdown of phosphatidylinositol 4,5-bisphosphate (PtdInsP_2) and enhanced labelling of phosphatidate (PA) and phosphatidylinositol (PtdIns). Lithium alone had little effect upon $^{32}\text{P}_i$ incorporation, but in combination with carbachol it greatly reduced the PtdIns labelling response to the agonist. Instead the label accumulated in a lipid identified as cytidine monophosphorylphosphatidate. There was also an enhancement of the PA labelling response to carbachol. These lithium-induced alterations in agonist-stimulated phospholipid metabolism were reversed if 10–30 mM-inositol was included in the incubation medium. Despite reduced PtdIns synthesis, lithium had relatively little effect on polyphosphoinositide labelling in stimulated cells. Resynthesis of polyphosphoinositides was monitored in acinar cells that had been stimulated with carbachol and then treated with atropine to block muscarinic receptors. Treatment with lithium during the carbachol-stimulation phase reduced the rate of phosphatidylinositol 4-phosphate synthesis, but had no significant effect upon PtdInsP_2 . The results suggest that an active inositol phosphatase pathway is essential to maintain intracellular inositol levels, but that PtdInsP_2 synthesis is not markedly reduced by a substantial fall in intracellular inositol. This implies a close control over the rates of PtdInsP_2 breakdown and resynthesis during agonist stimulation.

INTRODUCTION

Lithium has been widely used in the treatment of manic depressive disorders (Belmaker *et al.*, 1983) for many years, yet its precise mode of action remains obscure. Most current suggestions cannot readily explain the therapeutic effectiveness of serum lithium concentrations in the range 0.5–1.0 m-equiv./litre. An important contribution was made by Allison & Stewart (1971) who showed that lithium reduced brain inositol levels and further studies demonstrated that this was accompanied by a large increase in inositol 1-phosphate [$\text{Ins}(1)\text{P}$] (Sherman *et al.*, 1981). Lithium achieves this effect by potentially inhibiting $\text{Ins}(1)\text{P}$ phosphatase (Hallcher & Sherman, 1980) which cleaves D- $\text{Ins}(1)\text{P}$ obtained from inositol phospholipids and L- $\text{Ins}(1)\text{P}$ from the synthetic pathway *de novo* for inositol derived from glucose.

D- $\text{Ins}(1)\text{P}$ is the predominant isomer found in Li^+ -treated rats and its accumulation is blocked by atropine, suggesting that it is formed during agonist-stimulated inositol phospholipid breakdown (Sherman *et al.*, 1981).

In recent years several important advances have been made in understanding the role of inositol phospholipids in receptor signalling processes. It is now well established that activation of a broad spectrum of receptors stimulates a specific phospholipase C whose primary target is phosphatidylinositol 4,5-bisphosphate (PtdInsP_2) (Berridge, 1983; Downes & Michell, 1982; Downes & Wusteman, 1983; Aub & Putney, 1984; Berridge &

Irvine, 1984) leading to the formation of two potent intracellular signals: diacylglycerol (DG) remains in the membrane where it activates protein kinase C (Nishizuka, 1984), and inositol 1,4,5-trisphosphate (InsP_3) generates a Ca^{2+} signal by releasing Ca^{2+} from intracellular stores (see Berridge & Irvine, 1984, for a review).

Dephosphorylation of InsP_3 is an important process because it inactivates this potent intracellular signal and because it leads to recovery of free inositol which can be used for phosphatidylinositol (PtdIns) resynthesis. Signal inactivation is achieved by a highly specific phosphatase which cleaves the 5-phosphate to yield inositol 1,4-bisphosphate (InsP_2). Both membrane-bound and soluble activities have been described (Downes *et al.*, 1982; Storey *et al.*, 1984; Seyfred *et al.*, 1984). At least in liver, InsP_2 can be dephosphorylated at either the 1- or the 4-phosphate position by a soluble enzyme(s) which, unlike the InsP_3 5-phosphatase, is Li^+ -sensitive (Storey *et al.*, 1984). InsP phosphatase is also soluble and potentially inhibited by Li^+ (Hallcher & Sherman, 1980; Storey *et al.*, 1984).

This dephosphorylation pathway is, therefore, a complex process involving a cascade of specific enzymes. Whether the components of this system differ from one tissue to another is not known at present, but its sensitivity to Li^+ is widespread as evidenced by the broad spectrum of tissues that accumulate inositol phosphates during agonist stimulation when Li^+ is present (Berridge *et al.*, 1982). We have now assessed the importance of this phosphatase pathway in maintaining intracellular inositol

Abbreviations used: PtdIns , phosphatidylinositol; PtdInsP , phosphatidylinositol 4-phosphate; PtdInsP_2 , phosphatidylinositol 4,5-bisphosphate; PA, phosphatidate; CMPPA, cytidine monophosphorylphosphatidate; DG, diacylglycerol; InsP , inositol monophosphate; InsP_2 , inositol bisphosphate; InsP_3 , inositol trisphosphate.

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levels, and cellular responsiveness, using cholinergically stimulated rat parotid gland fragments and acinar cells as a model system.

MATERIALS AND METHODS

All radioisotopes were obtained from Amersham International. Bovine serum albumin (fraction V) and soybean trypsin inhibitor were from BDH. MEM amino acid supplement (alpha medium with L-glutamate) was from GIBCO and trypsin (type IX from porcine pancreas) and all phospholipid standards were from Sigma.

Tissue and cell preparations

The preparation of rat parotid gland slices has been described previously (Downes *et al.*, 1982). The medium used for all slice experiments was a modified Krebs-Henseleit buffer containing (mM): NaCl (118), KCl (5.6), MgSO_4 (1.16), KH_2PO_4 (1.18), CaCl_2 (2), NaHCO_3 (25), DL- β -hydroxybutyrate (5). The medium was gassed periodically with O_2/CO_2 (19:1).

Acinar cells were prepared in a basic incubation medium as above, but with the following differences. It contained 1 mM- CaCl_2 and was supplemented with 5 mM-glucose, 50 mM-sucrose, 2% (v/v) MEM amino acid supplement (adjusted to pH 7.4 with NaOH) and 0.5% (w/v) bovine serum albumin. The medium was gassed with O_2/CO_2 (19:1) immediately before adding the albumin. Cells were prepared exactly as described by Kanagasuntheram & Randle (1976) except that the time for the final incubation of tissue fragments with collagenase varied considerably from one experiment to another. Typically it was between 45 and 60 min and was terminated when 90% of the tissue was sufficiently dispersed to pass through the tip of a 5 ml Gilson pipette. Large fragments still remaining at this stage were removed by filtration through fine muslin before centrifuging the dispersed cells through a solution of 4% (w/v) bovine serum albumin in basic incubation medium. Cell viability, assessed by luciferin/luciferase assay for ATP, was comparable with other published results (Stanley & Williams, 1979). The final resuspension of dispersed acinar cells was in basic incubation medium plus 2% (w/v) bovine serum albumin.

Lipid labelling studies

In $^{32}\text{P}_i$ -labelling experiments the phosphate content of the incubation medium was reduced to one-fifth of normal to improve radioisotope incorporation into phospholipids. Incubations of parotid slices were exactly as described previously (Downes *et al.*, 1982). Briefly, 50 μl portions of 1 g-packed slices containing between 0.75 and 1.0 mg of protein were pipetted into Beckman Biovials containing 200 μl of medium and 5 μCi of $^{32}\text{P}_i$ or 1 μCi of [^3H]cytidine. When dispersed cells were used, the volume of the final cell suspension was adjusted to give approx. 0.5 mg of protein/50 μl of cell suspension. Drugs were added in 10 μl volumes from stock solutions in the basic incubation medium.

The incubations were terminated by adding 940 μl of chloroform/methanol/conc. HCl (40:80:1, by vol.) and lipids were extracted as described previously (Downes *et al.*, 1982). Phospholipids were separated by t.l.c. using oxalate-impregnated silica gel 60 plates (Merck) developed in chloroform/acetone/methanol/acetic acid/water

(40:15:13:12:8, by vol.) according to Jolles *et al.* (1981). ^{32}P -labelled phospholipids were located by autoradiography and radioactivity was determined by liquid-scintillation spectrometry. The identity of each phospholipid species was determined by co-chromatography with commercial standards stained with I_2 vapour. For detection of [^3H]cytidine-labelled phospholipids, each lane of the thin layer plate was cut into 5 mm strips and radioactivity in each strip was determined by liquid-scintillation spectrometry.

Inositol uptake

Inositol uptake into acinar cells was assessed using 1 ml incubations containing basic preparation medium, approx. 10^6 cells and 0.5 μCi of [^3H]inositol. The incubations were supplemented with various concentrations of unlabelled inositol. They were terminated by adding 4 ml of ice-cold medium followed by rapid filtration through Whatman GF/C filters and the filters were rinsed with 2 $\times$ 4 ml of ice-cold medium. Extracellular entrapped fluid was assessed with [^{14}C]inulin and found to be negligible. An alternative method, based on that of Prpic *et al.* (1982) involved rapid centrifugation of cells through a layer of Ficoll and gave essentially identical results.

$^{86}\text{Rb}^+$ efflux

Parotid gland slices from three rats were incubated with 1 μCi of $^{86}\text{Rb}^+$ in 2.5 ml of incubation medium for 45 min and excess label was removed by washing the slices in unlabelled incubation medium. An aliquot of the slices was used to determine total tissue $^{86}\text{Rb}^+$ at the start of the experiment and 200 μl samples of the remaining, prelabelled slices were transferred to a perfusion chamber (volume slightly greater than 200 μl) and superfused for 30 min at a rate of 2 ml/min to obtain a steady state basal efflux rate. The apparatus consisted of 12 such perfusion chambers that could be run in parallel, allowing four sets of incubation conditions to be assayed in triplicate for each experiment. When lithium was present it was introduced 10 min before application of carbachol. After addition of drugs to the superfusate, 1 min fractions were collected and radioactivity was determined by liquid-scintillation spectrometry. The results are expressed as %/k/min which is the rate release constant for $^{86}\text{Rb}^+$ determined by expressing the radioactivity in each 1 min fraction as a percentage of the total radioactivity in the tissue at the beginning of each 1 min collection period.

RESULTS

Effects of lithium and muscarinic receptor activation on $^{32}\text{P}_i$ -labelling of PtdIns cycle intermediates

Parotid gland acinar cells were labelled with $^{32}\text{P}_i$ for 30 min and then stimulated with a maximally effective dose of carbachol, which acts on muscarinic receptors in parotid gland, for 60 min (see Jacobson *et al.*, 1985). Prolonged exposure to carbachol caused a marked reduction in labelling of PtdInsP_2 (Table 1) with only modest changes in PtdInsP labelling. In most experiments the latter compound showed a small reduction in $^{32}\text{P}_i$ incorporation, but this was less consistent than the pronounced decrease in labelled PtdInsP_2 and occasionally there was no significant change. These results are similar to those arising from brief stimulation of muscarinic

Table 1. Effects of lithium and carbachol stimulation on phospholipid labelling

Parotid gland acinar cells were labelled with either $^{32}\text{P}_i$ or $[^3\text{H}]$ cytidine, as described in the Materials and methods section, for 30 min before adding carbachol (10^{-3} M) and incubating for a further 60 min. Li^+ (10 mM) was substituted for Na^+ in the incubation medium and was present throughout the incubation. The results are expressed as d.p.m./500 μg of cell protein and are the means $\pm$ S.E.M. for triplicate incubations in a single representative experiment, one of five experiments using $^{32}\text{P}_i$ and a single, separate experiment using $[^3\text{H}]$ cytidine. *Significantly different from control, $P < 0.05$; **significantly different from carbachol alone, $P < 0.05$.

Treatment group	$^{32}\text{P}_i$ incorporated into phospholipid species					$[^3\text{H}]$ Cytidine incorporated into CMPPA
	PA	PtdIns	PtdInsP	PtdInsP ₂	CMPPA	
Control	6177 $\pm$ 397	14204 $\pm$ 1408	4879 $\pm$ 215	10208 $\pm$ 626	229 $\pm$ 39	1292 $\pm$ 114
Carbachol (10^{-3} M)	24690 $\pm$ 1022*	56095 $\pm$ 2069*	3829 $\pm$ 546	3764 $\pm$ 286	409 $\pm$ 52	1037 $\pm$ 31
Li^+ (10 mM)	6825 $\pm$ 438	15284 $\pm$ 716	5147 $\pm$ 218	10560 $\pm$ 69	645 $\pm$ 124*	1859 $\pm$ 173
Carbachol + Li^+	33388 $\pm$ 2494**	27035 $\pm$ 3735**	3230 $\pm$ 89	3234 $\pm$ 89	6613 $\pm$ 1473**	22992 $\pm$ 559**

receptors in this tissue (Weiss *et al.*, 1982b). There was also a large, approx. 4-fold, increase in ^{32}P -labelled PA. Since both PA and the monoester phosphates of the polyphosphoinositides are labelled close to isotopic equilibrium with the cellular ATP pool in such experiments (Weiss *et al.*, 1982a,b) these changes in labelling approximate to alterations in the quantities of these compounds.

Carbachol induced a pronounced increase in $^{32}\text{P}_i$ incorporation into PtdIns. This results from increased turnover and is associated with a significant fall in the tissue content of PtdIns (Jones & Michell, 1974).

Lithium alone had no significant effect on lipid labelling, but it exerted marked effects on the ability of carbachol to enhance PtdIns turnover. The most noticeable effect of lithium was to cause a large accumulation of label in a lipid species which co-chromatographed with a CMPPA standard. At the same time there was a large reduction in the PtdIns labelling response to carbachol and the accumulation of PA was significantly greater when lithium was present. Carbachol-induced alterations in polyphosphoinositide labelling were not significantly affected by lithium. The results shown in Table 1 were reproduced in one other experiment using dispersed acinar cells and essentially identical results were obtained in three other experiments using parotid gland slices. Identification of CMPPA as the compound whose $^{32}\text{P}_i$ labelling increased dramatically when lithium and carbachol were present together was achieved using $[^3\text{H}]$ cytidine labelled cells (Table 1; final column). There was some background labelling from the origin to the position of the solvent front, but each lane displayed a peak of radioactivity in the position expected for CMPPA. This peak increased slightly when lithium was present alone, but increased dramatically (approx. 20-fold) when the combination of lithium and carbachol was present.

All the above changes in phospholipid labelling could be reversed if 10–30 mM-inositol was present in the incubation medium. This is illustrated in Fig. 1 which compares labelling of PtdIns and CMPPA in acinar cells stimulated with carbachol in the presence of 10 mM-lithium. There was a progressive reduction in the label accumulated in CMPPA with increasing inositol concentration, concomitant with an increase in PtdIns labelling. At 30 mM, inositol restored CMPPA labelling to the level

Fig. 1. Carbachol and lithium effects on PtdIns and CMPPA labelling: reversal by inositol in medium

Parotid gland acinar cells were incubated with $^{32}\text{P}_i$ for 30 min, carbachol (10^{-3} M) was added and the incubations continued for a further 60 min. Lithium (10 mM) and the indicated concentrations of inositol were present throughout. Each point is the mean of duplicate incubations (which varied up to 10%) within a single experiment, one of three giving essentially identical results. CMPPA: ●, carbachol + lithium; ○, carbachol alone. PtdIns: ▲, carbachol + lithium; △, carbachol alone.

observed if lithium was absent, and 10 or 30 mM-inositol was needed to restore the PtdIns labelling response. Using an iterative curve-fitting procedure, half-maximal recovery was determined as 0.82 ± 0.4 mM-inositol (PtdIns labelling) and 0.97 ± 0.3 mM-inositol (CMPPA) respectively. Inositol at 10 mM in the incubation medium did not significantly alter lipid labelling, either in the presence or absence of a maximally effective dose of carbachol (results not shown).

In one experiment the rate at which intracellular inositol levels decline in response to carbachol plus lithium challenge was examined. Clearly, accumulation of CMPPA is the most reliable index of declining inositol levels, and a time-course of carbachol-stimulated CMPPA labelling is shown in Fig. 2. During the first 5 min there is little accumulation of labelled CMPPA

Fig. 2. Uptake of inositol into parotid gland acinar cells

Parotid gland acinar cells were incubated with [^3H]inositol ($0.5 \mu\text{Ci}$) and various concentrations of unlabelled inositol in 1 ml of preparation medium for 15 min. The incubations were terminated and inositol uptake assessed as described in the Materials and methods section. Uptake was linear with time for at least 60 min (not shown). Each point is the mean of duplicate incubations (less than 5% variation), one of two experiments that gave similar results.

compared with appropriate controls. However, there is a sharp increase in this labelled compound between 5 and 15 min.

Inositol uptake

Accumulation of [^3H]inositol in parotid acinar cells is shown in Fig. 3. Uptake was linear over a wide range of inositol concentrations (the lowest concentrations tested are not shown in Fig. 3) and showed no sign of saturation up to 10 mM.

Effects of lithium upon acinar cell function

The secretory response to carbachol was monitored continuously by measuring the efflux of $^{86}\text{Rb}^+$ from preloaded parotid gland slices (Putney, 1976). The effect of 10^{-4}M -carbachol is shown in Fig. 4. Higher doses did not elicit a larger response and occasionally the response was reduced compared with that to 10^{-4}M -carbachol. The response was completely blocked by atropine (results not shown) and the time-course of Rb^+ efflux was similar to that reported by Putney (1976). However, lithium (10 mM) had no significant effect on either the basal efflux rate or the pulse of Rb^+ efflux elicited by carbachol.

Recovery of polyphosphoinositides following atropine blockade of muscarinic receptors

The rate of synthesis of polyphosphoinositides in inositol-depleted parotid acinar cells is shown in Fig. 5. The cells were first labelled with $^{32}\text{P}_i$ for 30 min and then stimulated with carbachol (10^{-3}M) for 45 min. A large excess of atropine (10^{-5}M) was then added to rapidly block muscarinic receptors and alterations in polyphosphoinositide labelling were monitored during the next 5 min.

Fig. 3. Time-course of CMPPA accumulation in lithium- and carbachol-treated parotid slices

Parotid gland slices were labelled with $^{32}\text{P}_i$ for 30 min before adding carbachol to some of the tubes and continuing the incubations for the times indicated. The results are means of triplicate incubations (up to 10% variation). $\circ$, Control; $\square$, carbachol (10^{-3}M); $\blacksquare$, carbachol + lithium (10 mM).

Fig. 4. Effects of lithium and inositol on carbachol-evoked Rb^+ efflux from rat parotid gland slices

Parotid gland slices were loaded with $^{86}\text{Rb}^+$ and superfused as described in the Materials and methods section. Carbachol (10^{-4}M) was added to the superfusing medium after 5 min. Fractions (1 ml) were collected and radioactivity determined. Each point is the mean result from three superfusion chambers run in parallel. Error bars are shown at the peak efflux rate, but have been omitted from the rest of the Figure to avoid confusion. $\blacksquare$, Carbachol (10^{-4}M) at 5 min; $\triangle$, carbachol at 5 min, lithium (10 mM) throughout; $\blacktriangle$, carbachol at 5 min, lithium (10 mM) and inositol (10 mM) throughout. The arrow marks the addition of carbachol to the perfusate, the time-lag being due to the dead-space in the system. $\%k/\text{min}$ is the rate release constant determined as described in the Materials and methods section.

The carbachol-induced fall in PtdInsP_2 labelling was slightly enhanced in the presence of lithium (this is similar to the result shown in Table 1, but the effect of lithium

Fig. 5 Resynthesis of polyphosphoinositides following atropine blockade of carbachol-stimulated acinar cells

Acinar cells were labelled with $^{32}\text{P}_i$ for 30 min before adding carbachol (10^{-3} M) and continuing the incubations for a further 45 min. Atropine (10^{-5} M) was then added (time 0 on the figure) and labelling of polyphosphoinositides was followed for the next 5 min. Upper panel, PtdInsP; lower panel, PtdInsP₂. ○, Control; ■, carbachol; ▲, carbachol + lithium (10 mM) during preincubation, atropine added at time 0. The results are means $\pm$ S.E.M. for triplicate incubations in a single experiment, one of three that gave similar results.

was not statistically significant). Upon addition of atropine there was a rapid recovery of labelled PtdInsP₂ back to the control value. This recovery phase was not significantly altered if lithium was present throughout the incubation period. By contrast, carbachol did not induce a significant change in PtdInsP labelling, but in the presence of lithium there was a significant drop in the radioactivity associated with this compound. A consistent

observation was that, following atropine blockade, PtdInsP labelling rose significantly above the control level. Lithium markedly reduced this enhanced PtdInsP labelling.

DISCUSSION

Acinar cell inositol homeostasis

The clearest result to emerge from these studies is that the combination of 10 mM-lithium and a maximal dose of carbachol leads to a rapid decline in intracellular free inositol levels. The most sensitive marker for this effect is the steep rise in CMPPA levels that results from this treatment. Thus inositol levels fall sufficiently to limit seriously the activity of CMPPA inositol phosphohydrolase between 5 and 15 min after the onset of the stimulus (Fig. 3). This effect can be reversed if rather high levels of inositol are available in the medium because parotid acinar cells appear to possess a low-affinity, apparently non-saturable inositol uptake mechanism (Fig. 2) similar to that of hepatocytes (Prpic *et al.*, 1982). Another potential source of intracellular inositol is via synthesis *de novo* from glucose, but this pathway is not generally considered to be important in tissues other than brain and testis (Spector & Lorenzo, 1975).

When acinar cells were bathed in a medium containing 0.1 mM-inositol (close to the highest levels found in serum: Sherman *et al.*, 1981) there was little, if any, protection from the dramatic fall in intracellular inositol resulting from agonist stimulation in the presence of lithium. This demonstrates that the inositol phosphatase pathway is essential for maintaining free inositol levels in intensely stimulated parotid. Furthermore, the activity of this pathway is sufficient to maintain inositol supply during stimulation for at least 60 min, since inositol up to 10 mM in the medium did not influence agonist-stimulated PtdIns labelling or CMPPA accumulation in the absence of lithium.

Effects of lithium on polyphosphoinositide levels

The marked effects of lithium on PtdIns resynthesis in agonist-stimulated cells was not transferred to the polyphosphoinositide pools. In most experiments there was a slightly larger fall in PtdInsP₂ and PtdInsP labelling due to carbachol when lithium was present, but combination of the data from five similar experiments and analysis by paired *t*-test demonstrated that these apparent effects of lithium were not statistically significant. However, previous work demonstrated that the decline in steady state polyphosphoinositide levels may not be an accurate index of the rate of formation of inositol phosphates and DG because these lipids turn over completely several times during even relatively brief periods of stimulation (Downes & Wusteman, 1983). Thus it is the rate of resynthesis of polyphosphoinositides that largely determines the rate of formation of second messengers. This was assessed in experiments in which inositol levels were first depleted during agonist stimulation in the presence of lithium, and rapid polyphosphoinositide resynthesis was monitored following atropine blockade of muscarinic receptors (Fig. 4). This experimental design revealed a significant deficiency in PtdInsP synthesis, yet despite this, PtdInsP₂ resynthesis was not significantly reduced in the inositol depleted cells. This result suggests that powerful homeostatic mechanisms

may prevail to ensure adequate supplies of PtdInsP₂ even when synthesis of PtdIns and PtdInsP are seriously diminished. The same conclusion has been drawn by Drummond & Raeburn (1984) who obtained very similar results using thyrotropin-stimulated GH₃ pituitary cells.

Effects of lithium on acinar cell function

In view of the above arguments it was not surprising to find that lithium did not reduce the secretory response to muscarinic stimulation of parotid gland slices. The Rb⁺ efflux response was rapid and desensitized in about 15 min, yet the secretory cells could maintain rapid PtdInsP₂ resynthesis even after 45 min exposure to carbachol plus lithium.

The significant, inositol-reversible, potentiation by lithium of PA accumulation observed in carbachol-stimulated cells suggests that some interconversion of CMPPA and PA may occur. Since PA can be dephosphorylated to DG this suggests that lithium may significantly raise DG levels in agonist-stimulated cells, as noted by Drummond & Raeburn (1984) for thyrotropin-stimulated GH₃ pituitary cells. Since DG formed during agonist-stimulated inositol phospholipid breakdown may serve to activate protein kinase C, this raises the interesting possibility that lithium may actually potentiate responses in some cells, although this should not affect the present results because Rb⁺ efflux is controlled by the Ca²⁺ signal and appears not to be influenced by activators of protein kinase C (Putney *et al.*, 1984).

Conclusions

Lithium treatment greatly reduces intracellular inositol supply in intensely stimulated parotid acinar cells, but has no detectable acute effect on the quiescent cells. However, the consequences of inositol depletion appear more complex than we at first thought (Berridge *et al.*, 1982). Firstly, the rate of PtdInsP₂ synthesis and, therefore, presumably the rate of InsP₃ and DG formation, can be maintained in the face of declining PtdIns and PtdInsP synthesis. However, long-term treatment with more modest doses of lithium (as in the management of manic depression) might lead to some remodelling of the PtdInsP₂-dependent signal generation system. Secondly, the observation that lithium can potentiate DG accumulation (Drummond & Raeburn, 1984) adds an additional complexity to the situation, although it remains to be seen whether lithium can enhance protein kinase C-mediated cellular responses. Nevertheless, the widespread occurrence of inositol phospholipid-dependent receptors in the brain (see Downes, 1983, for a review) and the sensitivity of Ins1P phosphatase to lithium may still hold an explanation for the unique effectiveness of lithium in the treatment of manic-depressive psychoses.

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