Lithium stimulates accumulation of second-messenger inositol 1,4,5-trisphosphate and other inositol phosphates in mouse pancreatic minilobules without inositol supplementation

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Previous studies showed that lithium, beginning at therapeutic plasma concentrations in the treatment of manic depression, increased the accumulation of second-messenger inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] in cerebral cortex slices of guinea pig and rhesus monkey [Lee, Dixon, Reichman, Moummi, Los and Hokin (1992) Biochem. J. **282**, 377–385; Dixon, Lee, Los and Hokin (1992) J. Neurochem. **59**, 2332–2335; Dixon, Los and Hokin (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 8358–8362]. These studies have now been extended to a peripheral tissue, mouse pancreatic minilobules. In the presence of carbachol, concentrations of lithium from 1 to 20 mM sharply and progressively increased the accumulation of Ins(1,4,5)P_3 and inositol 1,3,4,5-tetrakisphosphate, followed by a decrease. Assay of these inositol polyphosphates by either the prelabelling technique or mass assay gave similar results. Atropine quenching of cholinergically stimulated pancreatic minilobules led to a rapid disappearance of Ins(1,4,5)P_3. This disappearance was impeded by lithium. This suggested that the lithium-induced elevation in Ins(1,4,5)P_3 was due to inhibition of the 5-phosphatase and, on the basis of the markedly elevated concentrations of inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3] and inositol 1,4-bisphosphate in the presence of lithium, probably by feedback inhibition by these latter two compounds. An additional mechanism, i.e. a stimulatory effect of lithium on phospholipase C, cannot, however, be ruled out. The other reaction product of phospholipase C, inositol cyclic 1:2,4,5-trisphosphate, also increased in the presence of lithium. This may also be due to inhibition of the 5-phosphatase, which is the exclusive mechanism for removal of this compound. The effects of lithium on the accumulation of other inositol phosphates paralleled that of Ins(1,4,5)P_3, with the exception of inositol 3,4-bisphosphate, which decreased. This was presumably due to the inhibition of Ins(1,3,4)P_3 1-phosphatase by lithium. Unlike mouse cerebral cortex slices [Lee, Dixon, Reichman, Moummi, Los and Hokin (1992) Biochem. J. **282**, 377–385], inositol supplementation was not required to demonstrate lithium-stimulated Ins(1,4,5)P_3 accumulation in mouse pancreatic minilobules. This indicates that inositol depletion sufficient to impair lithium-stimulated Ins(1,4,5)P_3 accumulation does not occur in mouse pancreatic minilobules, even though an elevation of cytidine diphosphodiacylglycerol occurred, indicating some inositol depletion due to lithium. Elevation of Ins(1,4,5)P_3 by lithium may be a general phenomenon in the central nervous system and peripheral tissues under non-rate-limiting concentrations of inositol.

INTRODUCTION

We recently showed that lithium, at concentrations as low as 1 mM (which is a therapeutic plasma concentration in the treatment of manic depression or bipolar disorder), increased accumulation of inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] in slices of cerebral cortex of guinea pig, rabbit and monkey (a therapeutically relevant model for humans) (Lee et al., 1992; Dixon et al., 1992). In mouse and rat, increases were only seen if the incubation medium was supplemented with inositol. In monkey and guinea pig cerebral cortex slices, supplementation with inositol was not required for maximum stimulation of Ins(1,4,5)P_3 accumulation. A possible explanation for the species differences between rodents and higher mammals in the absence of inositol supplementation is that there is a relative deficiency of inositol in rodent brain slices, which is indicated by the following observations: (1) the inositol content of rat cerebral cortex is only one-half that of guinea pig cerebral cortex (Allison and Stewart, 1971; Sherman et al., 1986); (2) 80% is lost on incubation of slices; and (3) addition of 10 mM inositol to the incubation medium is required to restore *in vivo* levels of inositol (Sherman et al., 1986).

In the present study, we have examined the effects of lithium on the accumulation of Ins(1,4,5)P_3 and a variety of other inositol phosphates in mouse pancreatic minilobules stimulated with carbachol. Lithium, in a range of concentrations between 1 mM and 10 mM, progressively increased the accumulation of Ins(1,4,5)P_3. Atropine-quench experiments indicated that this was due, at least in part, to inhibition of its breakdown. Unlike cerebral cortex in this species, supplementation with inositol was not required for maximum stimulation of Ins(1,4,5)P_3 accumulation.

EXPERIMENTAL

Preparation, prelabelling and drug treatment of minilobules

Minilobules were prepared by collagenase digestion of mouse pancreas, as previously described (Sekar et al., 1987; Dixon and Hokin, 1987). After preparation, minilobules (2–3 ml gravity-packed volume) were incubated for 60 min in 6 ml of 'incubation

Abbreviations used: Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; Ins(1,3,4,5)P_4, inositol 1,3,4,5-tetrakisphosphate; Ins(c1:2,4,5)P_3, inositol cyclic 1:2,4,5-trisphosphate; Ins(1,3,4)P_3, inositol 1,3,4-trisphosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; Ins(1,4)P_2, inositol 1,4-bisphosphate; Ins(1,3)P_2, inositol 1,3-bisphosphate; Ins(3,4)P_2, inositol 3,4-bisphosphate; CDPDG, cytidine diphosphodiacylglycerol.

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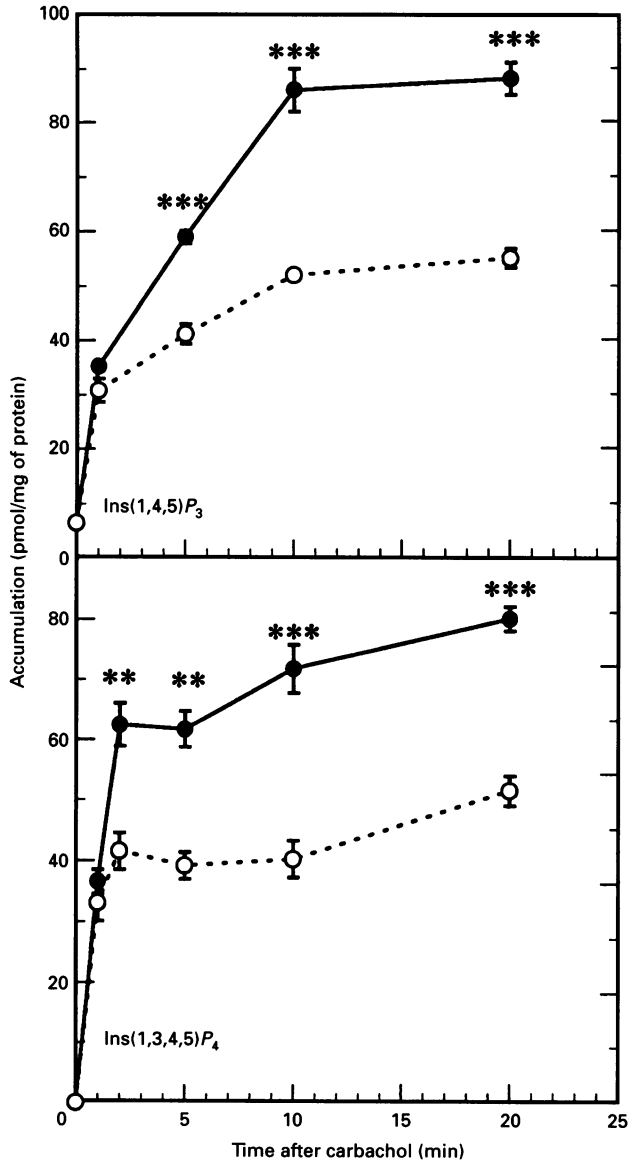

Figure 1 Time dependence of carbachol stimulation of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 accumulation in mouse pancreatic minilobules with and without lithium, as measured by mass assay

Unlabelled minilobules were preincubated for 10 min with (●) or without (○) LiCl (10 mM) and incubated for an additional 10 min with or without (zero time) carbachol (10 μM). Samples were quenched at various times by boiling. Preparation of unlabelled minilobules, quench, extraction, and binding assay of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 were as described in the Experimental section.

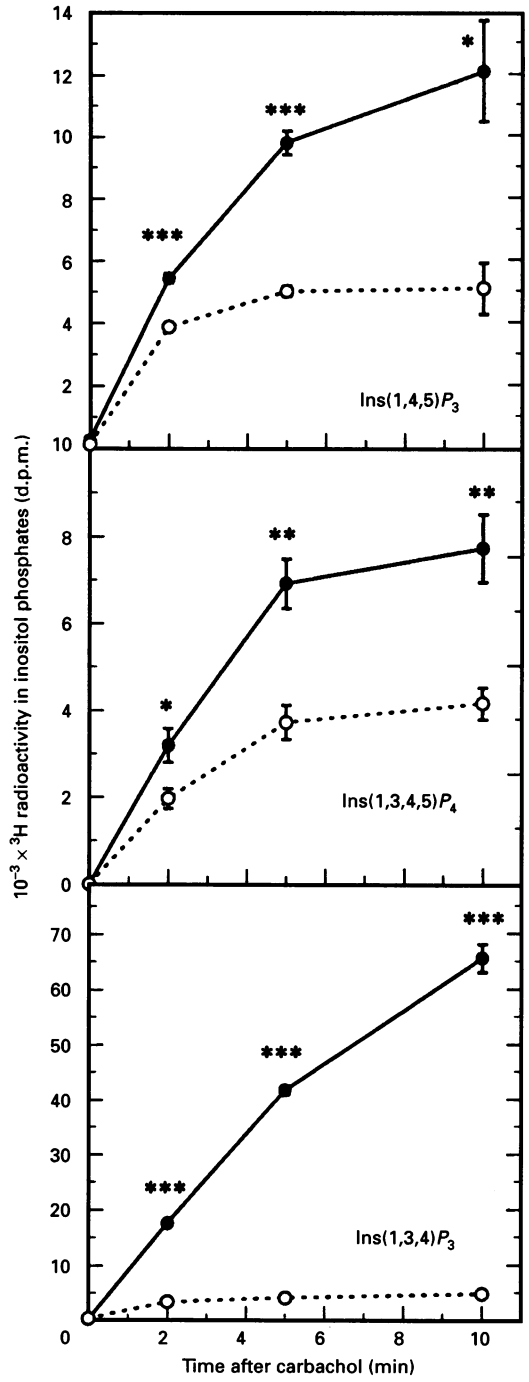

Figure 2 Time dependence of carbachol stimulation on the accumulation of two inositol trisphosphates and Ins(1,3,4,5)P_4 in mouse pancreatic minilobules with and without lithium, as measured by the prelabelling technique

[^{3}H]Inositol-prelabelled minilobules were preincubated for 10 min with (●) or without (○) LiCl (10 mM) and incubated an additional 10 min with or without carbachol (10 μM). Samples were quenched at various times by boiling. Preparation of minilobules, prelabelling, quench, extraction, and separation of inositol phosphates by h.p.l.c. were as described in the Experimental section.

medium' (Dixon and Hokin, 1984) with 200 μCi of [^{3}H]*myo*-inositol. Subsequently, label was removed by washing three times with 25 ml of incubation medium. The tissue suspension was separated into 12–24 equal portions and incubated with or without various concentrations of LiCl for 10 min. Carbachol was then added to some samples and the incubation continued for various times before quenching by boiling.

Unlabelled minilobules

Prelabelling was unnecessary when Ins(1,4,5)P_3 and inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] were determined by the binding assay. In these experiments, the incubation protocol used for prelabelling was used in the absence of label. Identical

Table 1 Comparison of the effects of lithium on accumulations of [^{3}H]Ins(c1:2,4,5)P_3 and [^{3}H]Ins(1,4,5)P_3 in carbachol-stimulated mouse pancreatic minilobules

Labelled minilobules were preincubated ± LiCl (10 mM) for 10 min, carbachol (10 μM) was then added and the incubation continued for 10 min before boiling-quench. [^{3}H]Ins(c1:2,4,5)P_3 and [^{3}H]Ins(1,4,5)P_3 were counted after separation by h.p.l.c. Quench, extraction and h.p.l.c. were as described in the Experimental section.

	[^{3}H]Ins(c1:2,4,5)P_3 (d.p.m.)				[^{3}H]Ins(1,4,5)P_3 (d.p.m.)			
	Control	Li^+ (10 mM)	P	Increase (%)	Control	Li^+ (10 mM)	P	Increase (%)
1	10418±469	13649±706	0.026	31.0	12129±543	20429±2047	0.005	68.4
2	5058±161	6628±138	< 0.001	31.0	6647±180	13522±245	< 0.001	103.4
3	7084±171	8732±504	0.019	23.3	8943±482	15903±1423	0.003	77.8

protocols were used so that conditions would be the same for both methods of quantification.

Boiling and extraction

Quenching and extraction by boiling was as previously described (Dixon and Hokin, 1989). Boiling-quench and extraction resulted in a combined yield of [^{3}H]Ins(1,4,5)P_3 and [^{3}H]inositol cyclic 1:2,4,5-trisphosphate {[^{3}H]Ins(c1:2,4,5)P_3} equal to the yield of [^{3}H]Ins(1,4,5)P_3 obtained after perchloric acid-quench, except for 5–10% of total [^{3}H]Ins(c1:2,4,5)P_3 hydrolysed to [^{3}H]Ins(2,4,5)P_3 (J. F. Dixon and L. E. Hokin, unpublished work). Boiling was used routinely to allow recovery and separation of cyclic inositol polyphosphates from non-cyclic inositol polyphosphates. Since acid extraction hydrolyses [^{3}H]Ins(c1:2,4,5)P_3 to [^{3}H]Ins(1,4,5)P_3 and [^{3}H]Ins(2,4,5)P_3 and since [^{3}H]Ins(c1:2,4,5)P_3 accumulates in amounts equal to up to 50% of [^{3}H]Ins(1,4,5)P_3 in pancreas after stimulation with carbachol (Dixon and Hokin, 1989), acid extraction gives overestimates of Ins(1,4,5)P_3 and abolishes Ins(c1:2,4,5)P_3 and other cyclic inositol phosphates.

H.p.l.c. of inositol phosphates

Inositol phosphates were separated by h.p.l.c. on a strong anion-exchange column with an $(NH_4)H_2PO_4$ gradient at pH 3.8, as previously described (Sastry et al., 1992; Lee et al., 1992).

Mass measurement of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 by receptor-binding assay

Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 were assayed by radiotracer displacement binding assays, as described by Donié and Reiser (1989). Preparation of binding protein was modified as follows: after all but the final homogenization of tissue or membranes, the homogenate was filtered through 150 μm polyethylene mesh (Spectra Mesh, Cole Parmer Instrument Co.). Binding protein from liver was used for Ins(1,4,5)P_3 determination. Ins(1,4,5)P_3-binding protein from liver has several advantages over that prepared from bovine adrenals: (a) fresh beef liver is plentiful and easy to obtain; (b) beef liver binding protein sediments easily and tightly during centrifugal separation from the reaction mixture; and (c) maximum specific binding is high, typically giving an increment of 3000 d.p.m. between 1 pmol and 5 pmol, and non-specific binding is well below 10%.

Statistical analysis

The figures illustrate representative experiments repeated two or more times and show the mean±S.E.M. from three or four replicates for each data point. The statistical significance of the effect of lithium was calculated using Student's t test and is displayed with asterisks as follows: *P value < 0.05, **P value ⩽ 0.01 and ***P value ⩽ 0.001. In Figures 1–3 and 7 the points represent incubation with and without lithium and the P value of the difference was calculated. In Figures 4–6 the P value was calculated for the difference between a control sample without lithium and samples with various concentrations of lithium.

RESULTS

Effects of lithium on the accumulation of inositol phosphates in the presence of carbachol

Figure 1 shows the effects of carbachol on the formation of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, as measured by mass assay. After a lag of 1 min following addition of carbachol, lithium stimulated the formation of both inositol polyphosphates. In the case of Ins(1,4,5)P_3, the stimulation levelled off after 10 min, at which point the concentration of the messenger was approximately double that of the control value. With Ins(1,3,4,5)P_4, stimulation plateaued after 2 min. With the exceptions of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, assay of all other inositol phosphates can only be done by the prelabelling technique. Figure 2 shows the effects of lithium on two inositol trisphosphates, as well as Ins(1,3,4,5)P_4, as measured by the prelabelling technique. The results with Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 were similar to those obtained by mass assay (note that in Figure 2 the maximum incubation time was 10 min). Lithium increased inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3] accumulation over 12-fold, while it stimulated Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 accumulations 2- to 3-fold. The considerable stimulation of Ins(1,3,4)P_3 accumulation by lithium has been shown before in pancreas (see Rana and Hokin, 1990). The formation of cyclic inositol phosphates in the presence of cholinergic agents was previously demonstrated in mouse pancreatic minilobules (Dixon and Hokin, 1987; Sekar et al., 1987). Table 1 shows, in three separate experiments, that there was a statistically significant 25–30% increase in Ins(c1:2,4,5)P_3 formation in the presence of lithium. However, this was only one-half to one-third of the increase in Ins(1,4,5)P_3. Possible interpretations of these results are deferred to the Discussion.

Figure 3 shows the effect of increasing concentrations of carbachol on the mass of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4. The accumulation of Ins(1,3,4,5)P_4 reached a maximum at 1×10^{-5} M carbachol, but the accumulation of Ins(1,4,5)P_3 continued to increase up to 1×10^{-3} M. This suggests that phosphorylation of Ins(1,4,5)P_3 was the rate-limiting reaction at higher concentrations of carbachol, rather than phosphodiesteratic cleavage of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. Previous studies have shown that phosphatidylinositol turnover continues to increase in pancreas as the acetylcholine concentration is

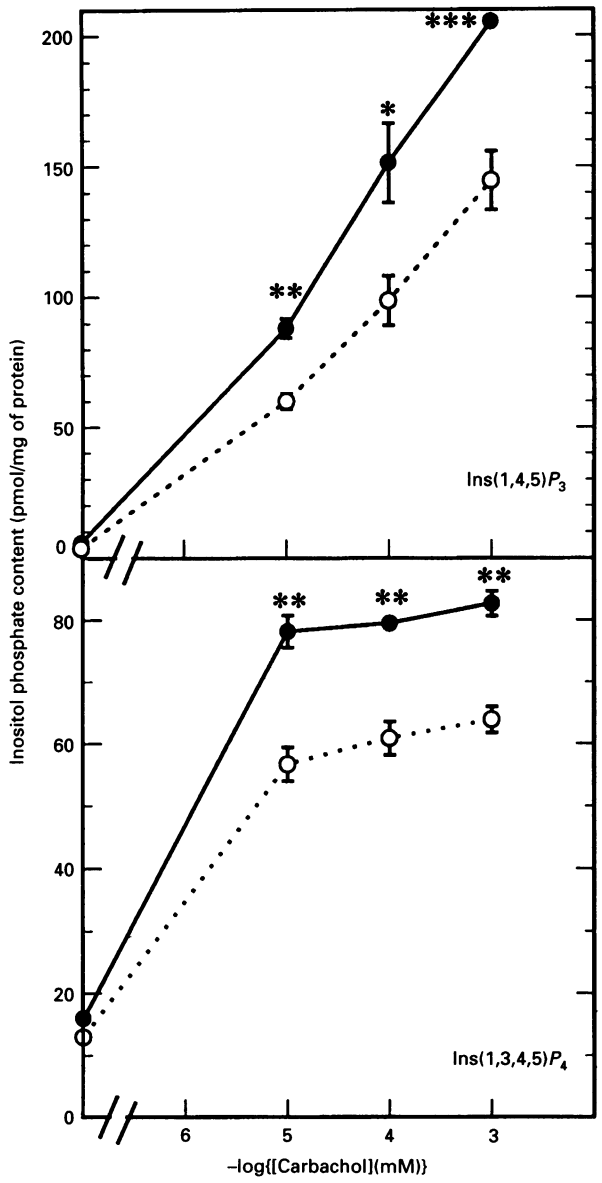

Figure 3 Effect of carbachol concentration on Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 mass in mouse pancreas minilobules with and without lithium

Unlabelled minilobules from mouse pancreas were incubated with (●) or without (○) LiCl (10 mM) for 10 min and incubated for an additional 10 min after the addition of various concentrations of carbachol. The reaction was quenched by boiling. Preparation of unlabelled minilobules, quench, extraction and binding assay of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 were as described under the Experimental section.

increased beyond 1×10^{-5} M carbachol equivalents (Hokin and Hokin, 1954).

Effect of lithium concentration on accumulation of Ins(1,4,5)P_3, Ins(1,3,4,5)P_4 and other inositol phosphates

Figures 4 and 5 show the effects of increasing concentrations of lithium on inositol phosphate accumulation, as measured by the prelabelling technique. Figure 4 shows the effects of 1–4 mM lithium on the accumulation of Ins(1,4,5)P_3, Ins(1,3,4,5)P_4 and several other inositol phosphates in the presence of carbachol. Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 increased progressively up to 4 mM lithium. There were significant increases in these inositol phosphates at 1 mM lithium, which is the mid-point in the therapeutic range in the treatment of manic depression. The inositol monophosphates, inositol 1,4-bisphosphate [Ins(1,4)P_2], and inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3], were increased considerably by lithium. Inositol 1,3-bisphosphate [Ins(1,3)P_2] was increased slightly, and inositol 3,4-bisphosphate [Ins(3,4)P_2] actually decreased. Figure 5 shows the effects of lithium concentrations up to 80 mM. Similar results were seen up to 10 mM lithium, as were seen in Figure 4, but with most of the inositol phosphates accumulation either plateaued or decreased at higher lithium concentrations. At higher concentrations, the dose–response curves for Ins(1,3,4,5)P_4, Ins(1,3,4)P_3, and Ins(1,4)P_2 paralleled that of Ins(1,4,5)P_3 in that after plateauing at 20 mM their levels fell. This is not surprising since Ins(1,4,5)P_3 is the ultimate precursor of all the others. The fall at these very high concentrations may be due to the trapping of sufficient inositol to depress levels of PtdIns(4,5)P_2 and thus Ins(1,4,5)P_3, as occurs in mouse cerebral cortex slices at lower lithium concentrations in the absence of inositol supplementation. It is of interest that Ins(3,4)P_2 decreased sharply at 10 mM lithium. This is presumably a result of inhibition of Ins(1,3,4)P_3 1-phosphatase by lithium, decreasing formation of Ins(3,4)P_2. Ins(4,5)P_2 was undetectable in the presence or absence of lithium, suggesting that an Ins(1,4,5)P_3 1-phosphatase was not present in pancreatic minilobules under the conditions of these experiments. Jenkinson et al. (1992) could not demonstrate Ins(1,4,5)P_3 1-phosphatase in broken preparations of rat brain.

Figure 6 shows the effects of increasing concentrations of lithium on Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 accumulation, as measured by mass assay. The percentage increase and the point of inflection are in close agreement with those in Figure 5, as measured by the prelabelling technique.

Effect of lithium on the disappearance of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 on atropine-quenching of cholinergically stimulated pancreatic minilobules

The lithium-induced elevation in Ins(1,4,5)P_3 could be due to stimulation of phospholipase C, inhibition of a phosphatase, or both. A possible inhibition of a phosphatase can be tested by atropine-quenching of cholinergically stimulated pancreatic minilobules in the presence and absence of lithium. On atropine-quenching the half-life ($t_{\frac{1}{2}}$) for disappearance of Ins(1,4,5)P_3 in the absence of lithium was 4 s, while that in the presence of lithium was 7 s (Figure 7). These differences were statistically significant and were reproducible in three separate experiments. Thus at least part, if not all, of the increase in Ins(1,4,5)P_3 concentration in the presence of lithium was due to inhibition of a phosphatase reaction.

Lithium decreases the steady-state concentration of inositol, as demonstrated by an elevation in cytidine diphosphodiacylglycerol (CDPDG), but not sufficiently to change its effect on Ins(1,4,5)P_3 levels.

Lithium, by its lowering of inositol concentrations in cerebral cortex slices, is known to increase steady-state levels of its reactant, CDPDG (Downes and Stone, 1986; Godfrey, 1989; Kennedy et al., 1990; Lee et al., 1992). Figure 8 shows a similar effect in mouse pancreatic minilobules incubated with 10 mM lithium. However, the decrease in inositol, as reflected by elevated CDPDG, did not lead to an inositol dependence for maximum accumulation of Ins(1,4,5)P_3 in the presence of lithium with and without carbachol (Table 2). Thus in mouse pancreatic mini-

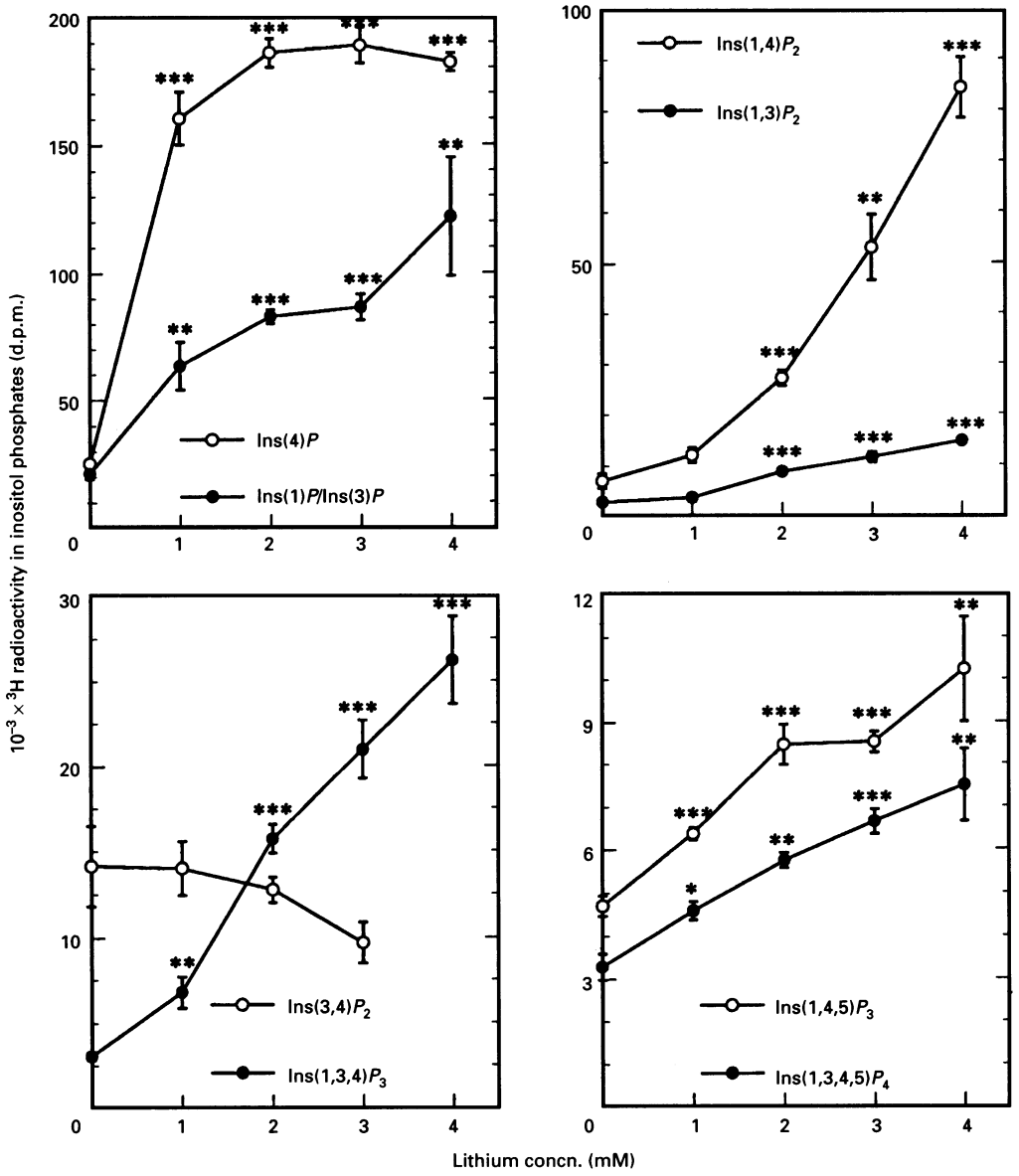

Figure 4 Effect of low concentrations of lithium on the carbachol-stimulated accumulation of [^{3}H]inositol phosphates in prelabelled mouse pancreatic minilobules

[^{3}H]Inositol prelabelled mouse pancreatic minilobules were incubated for 10 min with various concentrations of LiCl (0–4 mM) and then incubated for 10 min with carbachol (10 μM) before boil quench. Preparation and labelling of minilobules, quench, extraction, and separation of inositol phosphates by h.p.l.c. were as described in the Experimental section.

lobules, unlike mouse cerebral cortex slices, sufficient endogenous inositol is available in the presence of 10 mM lithium to permit a maximum effect of this ion on Ins(1,4,5)P_3 accumulation.

DISCUSSION

The response of Ins(1,4,5)P_3 to lithium in mouse pancreas is similar to that previously observed in guinea pig, rabbit and monkey cerebral cortex slices (Lee et al., 1992; Dixon et al., 1992, 1994) and in rat and mouse cerebral cortex slices supplemented with inositol (Lee et al., 1992). As reported here, in mouse pancreas, therapeutic concentrations of lithium increased Ins(1,4,5)P_3 content. The sensitivity to lithium in pancreas appeared to be greater in the therapeutic concentration range than in cerebral cortex slices of any species. The lithium stimulation was not increased by inositol supplementation. However, the level of CDPDG was increased by lithium in mouse pancreas, indicating that its reaction with inositol to form phosphatidylinositol was somewhat rate-limited but apparently not sufficiently so that addition of inositol increased the formation of Ins(1,4,5)P_3. Thus, in pancreas the lithium-induced increase in Ins(1,4,5)P_3 is independent of the well-documented inhibition of inositol recycling by lithium.

The salient observation reported here is that the stimulation of the accumulation by lithium of Ins(1,4,5)P_3 and of Ins(1,3,4,5)P_4 previously reported in cerebral cortex slices of guinea pig, rabbit and rhesus monkey (Lee et al., 1992; Dixon et al., 1992, 1994) can be extended to a peripheral tissue and may in fact be a

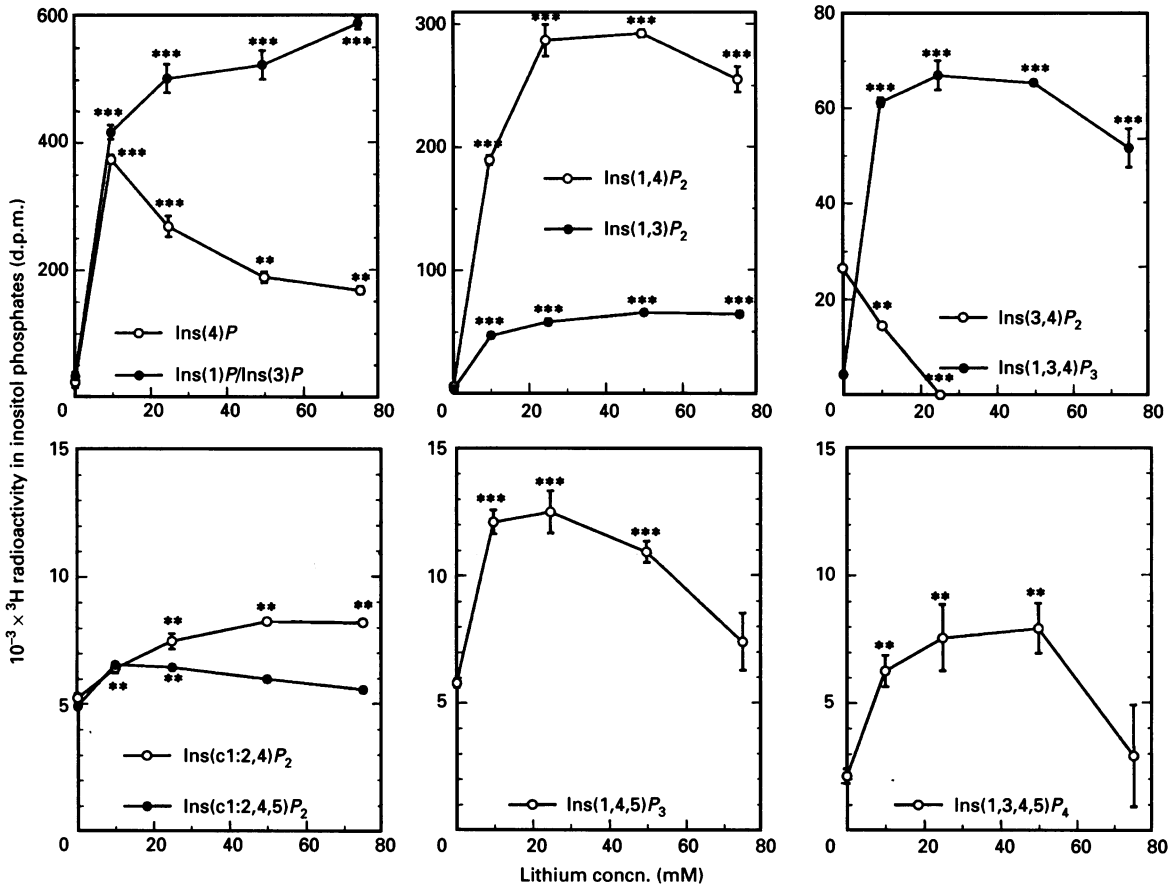

Figure 5 Effect of high concentrations of lithium on carbachol-stimulated accumulation of [^{3}H]inositol phosphates in prelabelled mouse pancreatic minilobules

[^{3}H]Inositol-prelabelled mouse pancreas minilobules were incubated for 10 min with various concentrations of LiCl (0–75 mM) and then incubated for 10 min with carbachol (10 μM) before boil quench. Preparation and labelling of minilobules, quench, extraction and separation of inositol phosphates by h.p.l.c. were as described in the Experimental section.

general phenomenon if inositol is not rate-limiting, as it is in cerebral cortex slices from mouse and rat. Thus lithium appears to enhance Ins(1,4,5)P_3 accumulation at a fundamental level in the central nervous tissue and peripheral tissue, involving muscarinic, serotonergic (Sastry et al., 1992) and glutamatergic (Dixon et al., 1994) systems in many species.

Rubin (1984) reported that 10 mM lithium increased the accumulation of an inositol trisphosphate fraction in caerulein-stimulated rat pancreatic acini, but at that time the presence in this fraction of Ins(1,3,4)P_3, which shows a striking increase in response to lithium (see Figures 2, 4 and 5) and which would confound the data, was not known.

The magnitude of the increase in the mass of Ins(1,4,5)P_3 in the presence of lithium is easier to assess in pancreas than in neural tissue. In neural tissue, there is a pool of Ins(1,4,5)P_3 (5–15 pmol/mg) which is not labelled by [^{3}H]inositol but which is demonstrable by mass assay in the absence of Ca^{2+} in the incubation medium. In the absence of Ca^{2+}, [^{3}H]Ins(1,4,5)P_3 was virtually undetectable (Dixon et al., 1992). This 'basal pool' does not exist in pancreas and therefore the results of prelabelling experiments closely parallel those of mass measurements.

We show here, by the use of atropine-quench experiments, that the lithium-induced elevation of Ins(1,4,5)P_3 in cholinergically stimulated pancreatic minilobules is due, at least in part, to an inhibition of the breakdown of these compounds. We found no inhibition of Ins(1,4,5)P_3 5-phosphatase in monkey brain homogenates up to 25 mM lithium, confirming an earlier study in platelets (Connolly et al., 1985). Ins(1,4,5)P_3 5-phosphatase is inhibited by its immediate reaction product, Ins(1,4)P_2, as well as by Ins(1,3,4)P_3 (the racemic form was used), with K_i values of 70–100 μM and 32 μM respectively (see Shears, 1989). Based on a protein content of 10% of the wet weight, and analysis of Ins(1,4,5)P_3 by mass assay, we can estimate that the concentration of Ins(1,4,5)P_3 in carbachol-stimulated minilobules in the presence of 10 μM lithium is 10–20 μM. Based on the relative radioactivities of Ins(1,3,4)P_3 and Ins(1,4)P_2, their concentrations are five and eight times the concentration of Ins(1,4,5)P_3, or 50–100 μM and 80–160 μM respectively. It is likely that under these conditions Ins(1,3,4)P_3 and Ins(1,4)P_2 would be inhibiting Ins(1,4,5)P_3 5-phosphatase to some extent and could account at least partly, if not wholly, for the lithium-induced inhibition of breakdown of this compound.

An alternative explanation for increased formation of Ins(1,4,5)P_3 and metabolites is stimulation of phospholipase C in intact cells. In this case, one would also expect a proportional increase in formation of Ins(c1:2,4,5)P_3, or an even greater increase, since Ins(c1:2,4,5)P_3 disappears much more slowly than Ins(1,4,5)P_3 on atropine-quenching of cholinergically stimulated pancreatic minilobules (Dixon and Hokin, 1989). There is only one mechanism for removal of Ins(c1:2,4,5)P_3, i.e. de-

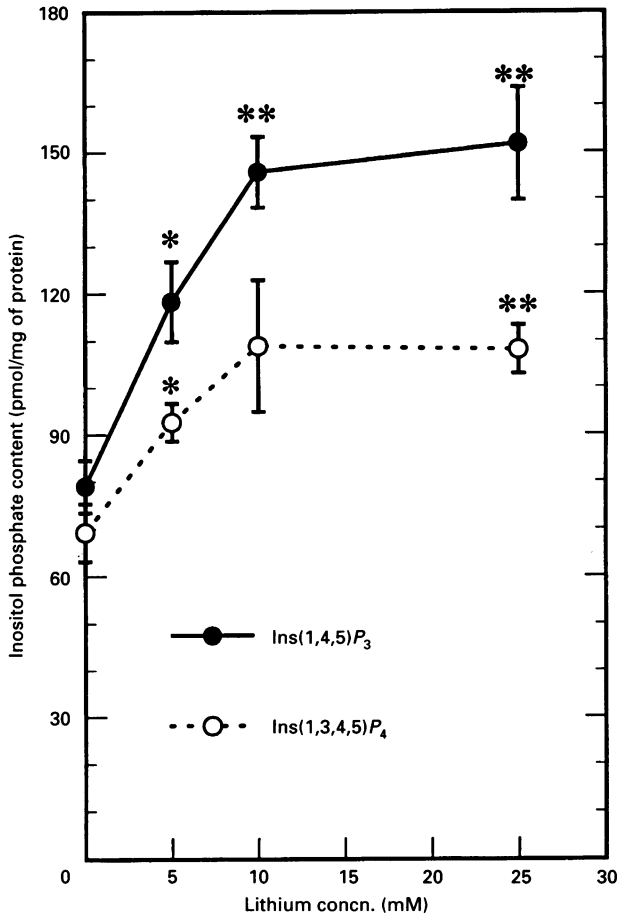

Figure 6 Effect of lithium concentration on carbachol-stimulated accumulation of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 mass in mouse pancreas minilobules

Unlabelled minilobules were preincubated for 10 min in the presence of various concentrations of lithium and then stimulated for 10 min with 10 μM carbachol. The reaction was quenched by boiling. Preparation of unlabelled minilobules, quench, extraction and binding assay of Ins(1,4,5)P_3 (●) and Ins(1,3,4,5)P_4 (○) were as described in the Experimental section.

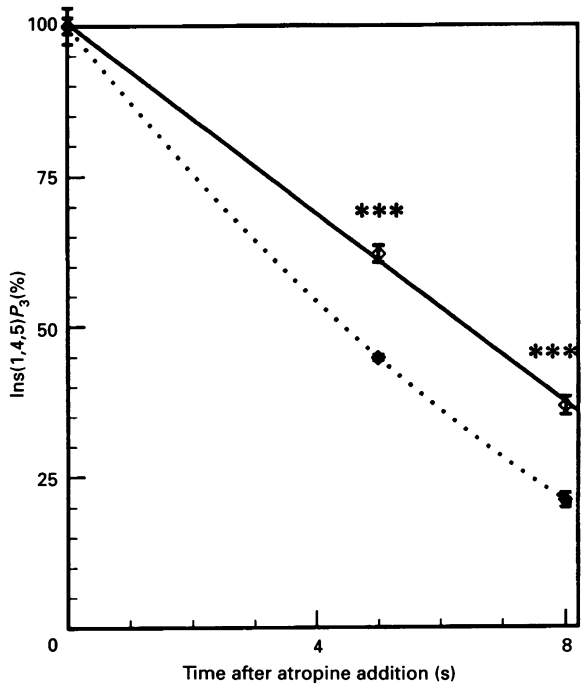

Figure 7 Effect of lithium on the rate of disappearance of Ins(1,4,5)P_3 after atropine blockade of carbachol-stimulated mouse pancreatic minilobules

Unlabelled minilobules were preincubated in single vessels either with (◆) or without (◇) LiCl (10 mM) for 10 min. Carbachol (10 μM) was then added and the incubation continued for 10 min. Separate aliquots were quickly taken for immediate boil-quenching or mixed with atropine for the times indicated before boil-quenching. Ins(1,4,5)P_3 mass in the water extracts was measured by the receptor binding assay. Preparation of unlabelled minilobules, quench, extraction and binding assay were as described in the Experimental section. The above data points are means ± S.E.M. of quadruplicate determinations. The P value of the difference between the lithium samples and the control samples was < 0.001 at both 5 and 8 s. Results are expressed as percentage of the appropriate zero-time control without atropine (± lithium). Control without lithium = 92.2 ± 1.5 pmol/mg. Control plus lithium = 157.6 ± 4.6 pmol/mg.

phosphorylation by a 5-phosphatase (Connolly et al., 1986), and one mechanism for its formation, i.e. diesteratic cleavage of PtdIns(4,5)P_2. The fact that lithium increased accumulation of Ins(1,4,5)P_3 more than that of Ins(c1:2,4,5)P_3, would tend to support inhibition of the 5-phosphatase rather than stimulation of phospholipase C. The lesser increase in Ins(c1:2,4,5)P_3 could be due to a lesser sensitivity to lithium-induced feedback inhibition by Ins(1,3,4)P_3 and Ins(1,4)P_2. An additional effect of lithium on the phospholipase C reaction cannot be ruled out, however. In fact, in acetylcholine-stimulated human neuroblastoma cells, where we also found increased Ins(1,4,5)P_3 accumulation in the presence of lithium (G. V. Los, I. Artemenko and L. E. Hokin, unpublished work) we could not demonstrate a retardation of Ins(1,4,5)P_3 breakdown on atropine clamping, suggesting that in this cell type the lithium enhancement may be due to an effect on phospholipase C action.

The lithium-induced increases in Ins(1,4,5)P_3 in the exocrine pancreas may have clinical significance since Ins(1,4,5)P_3 serves as a messenger in enzyme secretion in acinar cells (Streb et al., 1983). In fact, in a subset of lithium-treated patients, serum amylase was increased 2.5-fold (Tham et al., 1990). One of the major side effects of lithium treatment is diarrhoea, which may result from increased secretory activity in the gastrointestinal tract.

The existence of a lithium-sensitive Ins(1,4,5)P_3 1-phosphatase in mammalian pancreas, as has been demonstrated in *Dictyostelium discoideum* (Van Lookeren Campagne et al., 1988), might be at least a partial explanation for our results if this pathway for breakdown of Ins(1,4,5)P_3 were significant in pancreas. But we have not seen Ins(4,5)P_3 formation in the presence of lithium in our pancreas preparation. Jenkinson et al. (1992) could not directly demonstrate Ins(1,4,5)P_3 1-phosphatase in broken preparations of rat brain.

The role of phosphoinositides in signalling has been reviewed previously (Michell, 1989; Berridge and Irvine, 1989; Rana and Hokin, 1990; Berridge, 1993). The earlier studies showing an inhibition of Ins(1,4,5)P_3 accumulation by lithium (Kennedy et al., 1989, 1990; Nahorski et al., 1991; Lee et al., 1992) in rat and mouse cerebral cortex slices appear to be due to several factors: (1) rat cerebral cortex slices are uniquely deficient in inositol compared with other species (Allison and Stewart, 1971; Sherman et al., 1986; Lee et al., 1992). (2) In the presence of high concentrations of cholinergic agents, which break down unphysiologically large amounts of phosphatidylinositol, sufficient inositol is trapped in the form of inositol monophosphates, as

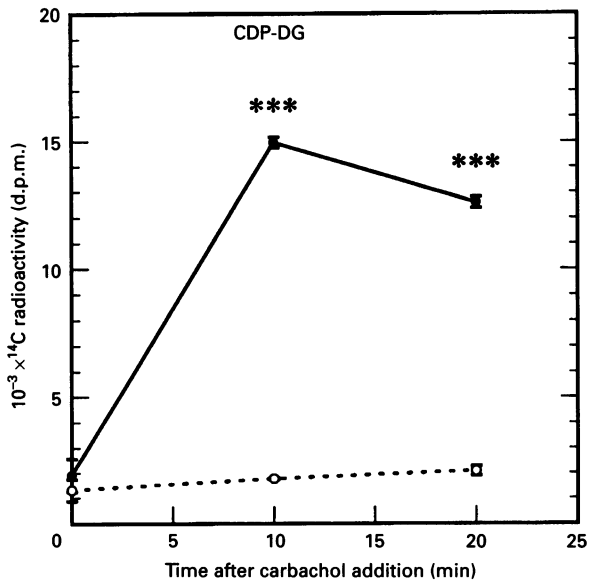

Figure 8 Effect of lithium on the accumulation of [^{14}C]CDPDG in mouse pancreatic minilobules at various times in the presence of carbachol

Minilobules were prelabelled with [^{14}C]cytidine, preincubated for 10 min with (●) or without (○) LiCl (10 mM), and then incubated for various times with carbachol (10 μM) before quenching by addition of 3.75 vol. of chloroform/methanol (1:2, v/v). The chloroform phase was removed and dried, and [^{14}C]CDPDG was counted for radioactivity.

well as Ins(1,4)P_2 and Ins(1,3,4)P_3, to lead to insufficient inositol to maintain normal levels of Ins(1,4,5)P_3 in rodent cerebral cortex slices unless the medium is supplemented with inositol. In fact, we have observed that in the absence of carbachol lithium stimulates accumulation of Ins(1,4,5)P_3 in mouse cerebral cortex slices without inositol supplementation (Dixon et al., 1994). (3) It has recently been shown that cholinergic agents markedly inhibit inositol uptake into astrocytoma cells (Batty et al., 1993). It is possible that a similar effect occurs in neuronal cells or that the effect on glial cells has secondary effects on neuronal cells to depress cellular inositol concentrations. The inhibitions seen *in vitro* in rat and mouse cerebral cortex slices do not occur in rat and mouse cerebral cortex *in vivo*, where inositol deficiency apparently does not occur. Using microwave fixation of brain tissue, a technique generally regarded as the most reliable for the measurement of heat-stable compounds *in vivo*, Whitworth et al. (1990) observed increases in Ins(1,4,5)P_3 in the forebrain of mice chronically treated with lithium and acutely stimulated with pilocarpine. Jope et al. (1992) showed no change in levels of Ins(1,4,5)P_3 in the brains of rats chronically treated with lithium, and in rats injected with pilocarpine there was an initial increase due to lithium, followed by a decrease (associated with convulsions). The later decrease is again probably due to trapping of large amounts of inositol in the form of inositol phosphates when in the presence of lithium plus excessive amounts of a cholinergic agent, a combination which produces convulsions in a species with limited amounts of inositol in its brain compared with higher species. In this connection, comparing different species in their susceptibility to lithium–pilocarpine seizures Bersudsky et al. (1994) found a parallel between susceptibility to lithium plus pilocarpine-induced seizures and decreases in brain cortical

Table 2 Effect of inositol supplementation of Ins(1,4,5)P_3 mass in mouse pancreatic minilobules, with and without carbachol in the presence of lithium

Unlabelled minilobules were preincubated for 15 min with LiCl (10 mM) with or without inositol (10 mM) and incubated for an additional 10 min with or without carbachol (10 μM) before boil-quench. Preparation of unlabelled minilobules, quench, extraction and binding assay of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 were as described in the Experimental section.

	Ins(1,4,5)P_3 (pmol/mg)	
	Control	(+) Carbachol (10 μM)
(−) Inositol	3.9±0.3	155±5
(+) Inositol (10 mM)	4.0±0.2	159±10

Ins(1,4,5)P_3 due to lithium plus carbachol, as previously found by us.

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REFERENCES

Allison, J. H. and Stewart, M. A. (1971) Nature New Biol. **233**, 267–268
Batty, I. H., Michie, A., Fennel, M. and Downes, C. P. (1993) Biochem. J. **294**, 49–55
Berridge, M. J. (1993) Nature (London) **361**, 315–325
Berridge, M. J. and Irvine, R. F. (1989) Nature (London) **341**, 197–205
Bersudsky, Y., Mahler, O., Kofman, O. and Belmaker, R. H. (1994) Eur. Neuropsychopharmacol, in the press
Connolly, T. M., Bross, T. E. and Majerus, P. W. (1985) J. Biol. Chem. **260**, 7868–7874
Connolly, M. T., Wilson, D. B., Bross, T. E. and Majerus, P. W. (1986) J. Biol. Chem. **261**, 122–126
Dixon, J. F. and Hokin, L. E. (1984) J. Biol. Chem. **259**, 14418–14425
Dixon, J. F. and Hokin, L. E. (1987) J. Biol. Chem. **262**, 13892–13895
Dixon, J. F. and Hokin, L. E. (1989) J. Biol. Chem. **264**, 11721–11724
Dixon, J. F., Lee, C. H., Los, G. V. and Hokin, L. E. (1992) J. Neurochem. **59**, 2332–2335
Dixon, J. F., Los, G. V. and Hokin, L. E. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 8358–8362
Donié, F. and Reiser, G. (1989) FEBS Lett. **254**, 155–158
Downes, C. P. and Stone, M. A. (1986) Biochem. J. **234**, 199–204
Godfrey, P. P. (1989) Biochem. J. **258**, 621–624
Hokin, M. R. and Hokin, L. E. (1954) J. Biol. Chem. **209**, 549–558
Jenkinson, S., Challiss, R. A. J. and Nahorski, S. R. (1992) Biochem. J. **287**, 437–442
Jope, R. S., Song, L. and Kolassa, K. (1992) Biol. Psychiatry **31**, 505–514
Kennedy, E. D., Challiss, R. A. J. and Nahorski, S. R. (1989) J. Neurochem. **53**, 1652–1655
Kennedy, E. D., Challiss, R. A. J., Ragan, C. I. and Nahorski, S. R. (1990) Biochem. J. **267**, 781–786
Lee, C. H., Dixon, J. F., Reichman, M., Moummi, C., Los, G. V. and Hokin, L. E. (1992) Biochem. J. **282**, 377–385
Michell, R. H. (1989) Curr. Opin. Cell Biol. **1**, 201–205
Nahorski, S. R., Ragan, I. C. and Challiss, J. R. A. (1991) Trends Pharmacol. Sci. **12**, 297–303
Rana, R. S. and Hokin, L. E. (1990) Physiol. Rev. **70**, 115–164
Rubin, R. P. (1984) J. Pharmacol. Exp. Ther. **231**, 623–627
Sastry, P. S., Dixon, J. F. and Hokin, L. E. (1992) J. Neurochem. **58**, 1079–1086
Sekar, M. C., Dixon, J. F. and Hokin, L. E. (1987) J. Biol. Chem. **262**, 340–344
Shears, S. B. (1989) Biochem. J. 260, 313–324
Sherman, W. R., Gish, B. G., Honchar, M. P. and Munsell, L. Y. (1986) Fed. Proc. Fed. Am. Soc. Exp. Biol. **45**, 2639–2646
Streb, H. R., Irvine, R. F., Berridge, M. J. and Schulz, I. (1983) Nature (London) **315**, 239–242
Tham, A., Johnson, L. and Mathe, A. A. (1990) Psychopharmacology **102**, 417–418
Van Lookeren Campagne, M. M., Erneux, C., Van Eijk, R. and Van Haastert, P. J. M. (1988) Biochem. J. **254**, 343–350
Whitworth, P., Heal, D. J. and Kendall, D. A. (1990) Br. J. Pharmacol. **101**, 39–44

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