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The effects of lithium on platelet phosphoinositide metabolism

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The effects on phosphoinositide metabolism of preincubation of platelets for 90 min with 10 mM-Li^+ were studied. Measurements were made of [^{32}P]phosphate-labelled phosphoinositides and of [^{3}H]inositol-labelled inositol mono-, bis- and tris-phosphate (Ins$P$, InsP_2 and InsP_3). (1) Li^+ had no effect on the basal radioactivity in the phosphoinositides or in InsP_2 or InsP_3, but it caused a 1.8-fold increase in the basal radioactivity in Ins$P$. (2) Li^+ caused a 4-, 3- and 2-fold enhanced thrombin-induced accumulation of label in Ins$P$, InsP_2 and InsP_3 respectively. Although the elevated labelling of InsP_2 and InsP_3 returned to near-basal values within 30–60 min, the high labelling of Ins$P$ did not decline over a period of 60 min after addition of thrombin to Li^+-treated platelets, consistent with inhibition of Ins$P$ phosphatase by Li^+. The effect of Li^+ was not due to a shift in the thrombin dose–response relationship; increasing concentrations of thrombin enhanced the initial rate of production of radiolabelled inositol phosphates, whereas Li^+ affected either a secondary production or the rate of their removal. (3) The only observed effect of Li^+ on phosphoinositide metabolism was a thrombin-induced decrease ($P < 0.05$) in labelled phosphatidylinositol 4-phosphate in Li^+-treated platelets; this suggests an effect on phospholipase C. (4) Li^+ enhanced ($P < 0.05$) the thrombin-induced increase in labelled lysophosphatidylinositol, suggesting an effect on phospholipase A_2. (5) It is concluded that Li^+ inhibits Ins$P$ phosphatase and has other effects on phosphoinositide metabolism in activated platelets. The observed effects occur too slowly to be the mechanism by which Li^+ potentiates agonist-induced platelet activation.

INTRODUCTION

With many types of cells, including platelets, the hydrolysis of phosphoinositides may be a primary event in agonist-induced activation [for reviews, see Berridge (1984) and Nishizuka (1984)]. Thus agonists cause an accelerated turnover of the phosphoinositides, and some of the products of the phospholipase C-catalysed hydrolysis of phosphoinositides may have second-messenger roles. For example, diacylglycerol has been shown to activate protein kinase C (Takai *et al.*, 1979), and InsP_3 has been reported to mobilize intracellular Ca^{2+} in permeabilized cells (Streb *et al.*, 1983). Either of these, or the combination of the two, could constitute a signal arising from a receptor-linked activation of phosphoinositide turnover.

Li^+ causes a decrease in the content of *myo*-inositol in rat brain (Allison & Stewart, 1971) and an increase in that of Ins$P$ (Allison *et al.*, 1976; Sherman *et al.*, 1981), a consequence of inhibition of Ins$P$ phosphatase (Hallcher & Sherman, 1980). The effects of Li^+ on phosphoinositides are more pronounced after addition of agonists; Berridge *et al.* (1982) demonstrated that Li^+ could effectively amplify the agonist-induced phosphoinositide response, measured as the accumulation of Ins$P$, of certain tissues. An enhanced agonist-induced accumulation of InsP_2 and InsP_3 (Drummond *et al.*, 1984; Thomas *et al.*, 1984) suggests that Li^+ might also inhibit phosphatases for these intermediates, but with cell-free systems such inhibition is not apparent (Seyfred *et al.*, 1984; Connolly *et al.*, 1985). The reported enhancement by Li^+ of agonist-induced diacylglycerol production (Drummond & Raeburn, 1984) cannot be attributed directly to inhibition of a phosphatase, suggesting still more extensive effects of Li^+ on agonist-accelerated phosphoinositide metabolism.

If Li^+ enhances the agonist-induced accumulation of these intermediates, and if any of the intermediates acts as a second messenger, Li^+ might potentiate the responses of cells to agonists. It has, in fact, been reported that prolonged incubation of human platelets in plasma containing added Li^+ leads to potentiation of agonist-induced aggregation, secretion and thromboxane synthesis (Imandt *et al.*, 1977, 1980, 1981).

To gain insight into the role of phosphoinositide metabolism in stimulus–response coupling in platelets, we have examined the effects of Li^+ on phosphoinositide metabolism in resting platelets and in platelets activated by thrombin.

EXPERIMENTAL

Materials

Purified human α-thrombin was given by Dr. John W. Fenton II, Division of Laboratories and Research, New York State Department of Health, Albany, NY, U.S.A. *myo*-[2-^{3}H(n)]inositol was from American Radiolabeled Chemicals, St. Louis, MO, U.S.A.; [^{32}P]P_i and L-*myo*-[U-^{14}C]inositol 1-phosphate were from Amersham, Northbrook, IL, U.S.A. T.l.c. plates (Silica HL) were from Analtech, Newark, DE, U.S.A., and ion-exchange resin AG1-X8 (200–400 mesh; formate form) was from Bio-Rad, Richmond, CA, U.S.A.

Abbreviations used: PtdIns, PtdIns4P and PtdIns4,5P_2, phosphatidylinositol and its 4-phosphate and 4,5-bisphosphate derivatives; Ins$P$, InsP_2 and InsP_3, inositol mono-, bis- and tris-phosphates; lyso-PtdIns, lysophosphatidylinositol.

Platelet preparation

Platelets were prepared from 200 ml of blood collected into acid/citrate/dextrose (Aster & Jandl, 1964) anticoagulant by venipuncture of healthy donors who had taken no drug during the previous 2-week period. Platelet-rich plasma, obtained by centrifugation of the blood at 300 *g* for 20 min at room temperature, was centrifuged at 1000 *g* for 20 min to recover platelets. The platelets were resuspended in 10 ml of modified Ca^{2+}-free Tyrode–Hepes buffer (134 mM-NaCl/12 mM-$NaHCO_3$/2.9 mM-KCl/0.34 mM-Na_2HPO_4/2 mM-$MgCl_2$/5 mM-Hepes/5 mM-glucose/1 mM-EGTA, adjusted to pH 7.4) containing either [^{3}H]inositol (70 μCi/ml) or [^{32}P]P_i (50 μCi/ml). After incubation for 2 h at 37 °C, the platelets were collected by centrifugation (1000 *g*, for 20 min), washed in 100 ml of the above buffer and finally resuspended in the same buffer but without EGTA and with 0.02 unit of apyrase/ml and 2 mg of albumin/ml (both from Sigma Chemical Co., St. Louis, MO, U.S.A.). This final suspension contained 4×10^8–6×10^8 platelets/ml. For studies of the effects of Li^+, platelets were incubated with an additional 10 mM-LiCl or 10 mM-NaCl (control) for 90 min.

Activation of platelets with thrombin

Reactions were at 37 °C with 1 ml platelet suspension in a 5 ml cuvette with a 3 mm stirring bar driven by a magnet at 1100 rev./min. Reactions were stopped by addition of the extraction solution directly to the cuvette. Thus each time point represents a separate incubation of a sample of a stock platelet suspension. This method was chosen over the withdrawal of samples at intervals from a single incubation, because it allowed precision of time and it avoided sampling error caused by changes (e.g. aggregation) during the incubation.

Measurement of labelled inositol phosphates

Labelled inositol phosphates from phosphoinositides pre-labelled with [^{3}H]inositol were measured by extraction with trichloroacetic acid and separation of the inositol phosphate by ion-exchange chromatography. Extraction was by addition of an equal volume of ice-cold 10% (w/v) trichloroacetic acid; the contents of the cuvette were then transferred to a centrifuge tube, and the cuvette was rinsed with 0.5 ml of 5% trichloroacetic acid, which was also added to the centrifuge tube. After centrifugation for 10 min at 1000 *g*, the supernatant solution was removed and extracted with 10 ml of diethyl ether to remove trichloroacetic acid. The aqueous phase, containing the inositol phosphates, was adjusted to pH 7.2 and diluted to 5 ml with water before application to an AG1-X8 ion-exchange column for separation of the inositol phosphates as described by Downes & Michell (1981). Elutions were with 16 ml of water (inositol), 16 ml of 0.2 M-ammonium formate/0.1 M-formic acid (Ins*P*), 16 ml of 0.4 M-ammonium formate/0.1 M-formic acid (InsP_2) and 16 ml of 1 M-ammonium formate/0.1 M-formic acid (InsP_3); 8 ml fractions were collected and counted for radioactivity in 10 ml of Hydrofluor scintillant (National Diagnostics). Over 90% recovery was obtained with [^{14}C]Ins*P* (Amersham) or [^{32}P]InsP_2 and [^{32}P]InsP_3 prepared from human red blood cells as described by Downes & Michell (1981).

Measurement of labelled phosphoinositides

The [^{32}P]phosphate-labelled phosphoinositides were extracted and analysed essentially as described by Billah & Lapetina (1982). Platelet suspensions (1 ml) were extracted with 3.6 ml of chloroform/methanol/HCl/100 mM-EDTA (100:200:2:3, by vol.). The phases were separated by addition of 1.2 ml of chloroform followed by 1.2 ml of 2 M-KCl. The lower phase (chloroform) was transferred and combined with a 4 ml chloroform wash of the upper phase. These combined chloroform phases were dried under N_2, and the lipids were dissolved in 60 μl of chloroform. The lipids were separated by t.l.c. on Silica HL plates impregnated with 1% potassium oxalate containing 2 mM-EDTA. The chromatograms were developed with chloroform/methanol/4 M-NH_3 (9:7:2, by vol.). It has been reported that with this chromatographic system an unidentified phospholipid can represent as much as one-third of the total radioactivity in the PtdIns4*P* spot, but that the amount of the contaminating radioactivity is not affected by activation of the platelets with thrombin (Tysnes *et al.*, 1985). After location of the lipids by autoradiography, spots were scraped off the plates and counted for radioactivity in 5 ml of Ultrafluor (National Diagnostics). For each time point, triplicate samples of the platelet suspension were extracted and chromatographed; the average of these three measurements was used as a single measurement for the statistical analysis.

RESULTS

Potentiation of platelet activation by Li^+

We confirmed the previous reports (Imandt *et al.*, 1977, 1980) that addition of 10 mM-Li^+ to human citrated platelet-rich plasma for 90 min potentiated platelet aggregation and secretion induced by ADP or the bivalent-cationophore A23187. The potentiation was slight; it was more obvious at agonist concentrations that gave biphasic aggregation, where just a slightly higher concentration of agonist causes a qualitatively different response. We also observed that Li^+ slightly potentiated the response to thrombin of platelets suspended in plasma-free buffered solutions. We were, however, unable to observe consistent potentiation after the prolonged incubations required to prelabel platelets for the measurements of phosphoinositide metabolism.

Thrombin-induced accumulation of [^{3}H]inositol phosphates

The time courses of thrombin-induced accumulation of inositol phosphates in human platelets have been measured as mass (Rittenhouse & Sasson, 1985) or as radioactivity (Watson *et al.*, 1984; Siess, 1985), with somewhat different results; the mass of InsP_3 reached a maximum by 15 s and returned to basal values within 2 min, whereas the labelled InsP_3 remained elevated after 2 min. Our results with platelets preloaded with labelled inositol, shown in Fig. 1 for three concentrations of thrombin, are similar to the results of other radioactivity measurements (Watson *et al.*, 1984; Siess, 1985). The data are plotted in two ways. The plots on the left permit analysis of the thrombin-concentration dependence for each of the inositol phosphate compounds, whereas the plots on the right facilitate comparison of the time courses and magnitudes of changes in radioactivity

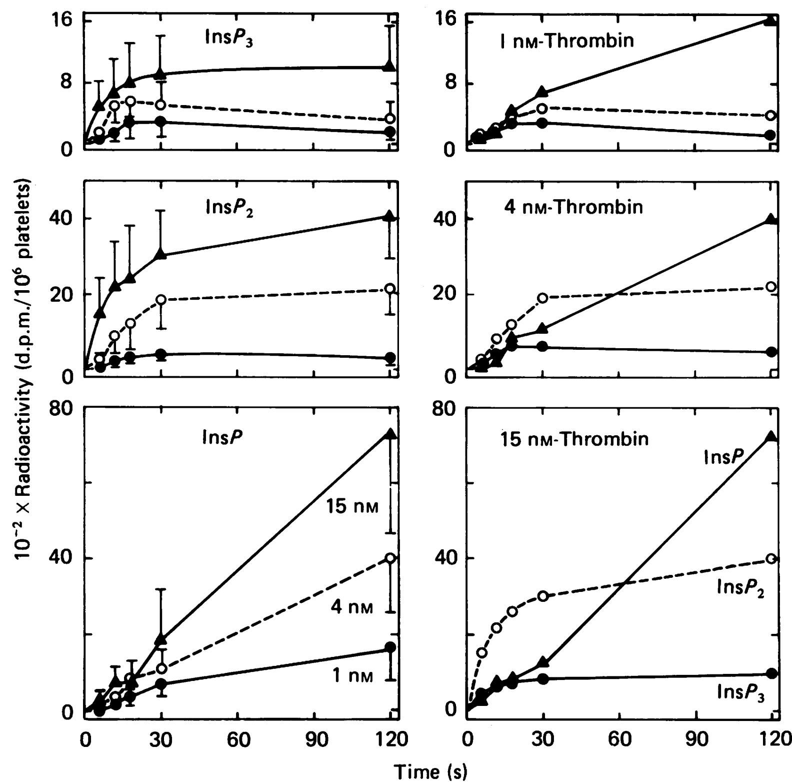

Fig. 1. Time courses of thrombin-induced changes in amounts of labelled inositol phosphates

Platelets prelabelled with [3H]inositol were activated with 1 nM-, 4 nM- or 15 nM-thrombin, and extracts were analysed for labelled inositol phosphates as described in the Experimental section. The data (means ± S.D. for three separate experiments) are expressed as d.p.m./10^6 platelets above the zero-time values, which were: InsP_3, 264 ± 28; InsP_2, 616 ± 144; Ins$P$, 2270 ± 885. The graphs on the left show radioactivity in InsP_3, InsP_2 and Ins$P$ after addition of (●) 1 nM-, (○) 4 nM- or (▲) 15 nM-thrombin. The graphs on the right show radioactivity in InsP_3 (●), InsP_2 (○) and Ins$P$ (▲) after addition of 1 nM-, 4 nM- or 15 nM-thrombin.

among the three intermediates. Accumulation of label in Ins$P$ was essentially linear for at least 2 min, but the label in InsP_2 and InsP_3 approached a plateau within 30 s. With higher concentrations of thrombin, the changes were greater, but the shapes of the time courses were unchanged.

Effect of Li^+ on the thrombin-induced accumulation of [3H]inositol phosphates

There are two ways that Li^+, through inhibition of Ins$P$ phosphatase, might make platelets more responsive to agonists. A higher steady-state basal concentration of a second messenger could make platelets more sensitive to activation, or the achievement of a higher concentration of the messenger in response to the agonist could make the agonist more effective.

Preincubation of [3H]inositol-loaded platelets with 10 mM-Li^+ had no effect on the basal radioactivity in InsP_2 or InsP_3, but it did cause a nearly 2-fold increase in radioactivity in Ins$P$ [385 ± 111 c.p.m. ($n = 16$) after 90 min with 10 mM-Li^+ versus 215 ± 69 c.p.m. ($n = 20$) for control platelets]. Similarly, Siess (1985) reported that incubation of [3H]inositol-loaded platelets for 60 min with 10 mM-Li^+ was accompanied by a 5-fold increase in labelled Ins$P$.

Preincubation with Li^+ also caused an enhanced thrombin-induced accumulation of radioactivity in two of the inositol phosphate intermediates. The time courses of these accumulations in response to 4 nM- or 15 nM-thrombin in the presence or absence of 10 mM-Li^+ are compared in Fig. 2; the time course for a still higher concentration of thrombin was included in this experiment to contrast the Li^+ effect with the effect of increasing the concentration of thrombin. Although there was little effect of Li^+ on thrombin-induced increases in labelled Ins$P$, there were substantial effects on the accumulation of labelled InsP_2 and InsP_3. The effect of Li^+ was apparent only at the later time points; with Li^+ the initial increases in labelled InsP_2 and InsP_3 were the same as for controls, but a higher plateau was achieved (InsP_3) or the labelling continued to increase for the 2 min period (InsP_2). In contrast, when the response was enhanced by activation with a higher concentration of thrombin, each intermediate was affected equally, and the shapes of the time course plots were unaffected. These results suggest that the concentration of thrombin determined the initial rate of incorporation of label into the inositol phosphate intermediates, whereas Li^+ affected either a secondary incorporation or the rate of removal. The latter would be consistent with inhibition of the phosphatases by Li^+.

Since the failure of Li^+ to affect appreciably the thrombin-induced accumulation of labelled Ins$P$ was surprising, in view of its reported inhibition of Ins$P$ phosphatase (Hallcher & Sherman, 1980), we examined the effects of longer incubation with thrombin (Fig. 3). It was then clear that in fact the effect of Li^+ was by far

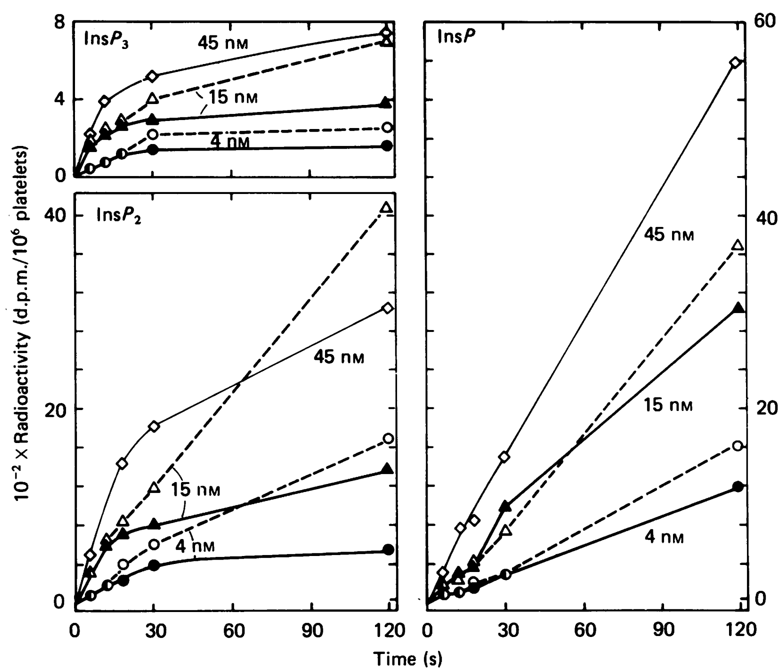

Fig. 2. Effect of Li^+ on time courses of initial thrombin-induced changes in $InsP_3$, $InsP_2$ and InsP

The experiments were as described for Fig. 1 with platelets incubated for 90 min at 37 °C after addition of either 10 mM-NaCl (solid symbols and continuous lines) or 10 mM-LiCl (open symbols and broken lines). Platelets were activated with (●, ○) 4 nM-, (▲, △) 15 nM- or (◇) 45 nM-thrombin. The data (means of five separate experiments, with S.D. values 50–160% of the means) are expressed as d.p.m./10^6 platelets above the zero-time values, which were for $InsP_3$, $InsP_2$ and InsP 252 ± 100, 308 ± 189 and 922 ± 233 for control (NaCl) platelets and 284 ± 84, 342 ± 149 and 1540 ± 451 for Li^+-treated platelets.

the greater on accumulation of label in InsP, but that substantial differences were observed only after 5 min. With the control platelets thrombin caused a quick increase in the label in each intermediate, presumably owing to activation of phospholipase C, followed by a slower return to nearly basal values, presumably reflecting cessation of production and removal of the products by the action of the phosphatases. Whereas Li^+ enhanced the thrombin-induced accumulation of labelled $InsP_3$ and $InsP_2$, the return toward basal values was unaffected. In contrast, Li^+ completely blocked the removal of labelled InsP (Fig. 3) and caused an equivalent and parallel decrease in labelled inositol (results not shown). These data suggest that the major effect of Li^+ was to inhibit InsP phosphatase. The enhanced accumulation of labelled $InsP_2$ and $InsP_3$ could be due either to partial inhibition of their phosphatases or to greater production.

Effect of Li^+ on thrombin-induced changes in [^{32}P]phosphoinositides

The effect of Li^+ on phosphoinositides was analysed with [^{32}P]phosphate-prelabelled washed platelets (Fig. 4). The thrombin-induced decrease in labelled PtdIns contrasts with other reports (Holmsen *et al.*, 1981, 1984) of substantial increases in labelled [^{32}P]PtdIns 2 min after addition of thrombin. The discrepancy appears to be due to different conditions for loading labelled phosphate. When loading was for 60 min with platelets resuspended in acid/citrate/dextrose-plasma, a widely used condition, we found that only 2% of the phosphoinositide label was in PtdIns. This indicates little incorporation of label into the diester phosphate, and is consistent with little turnover of phosphoinositides but rapid exchange between ATP and 4- and 5-phosphate groups of PtdIns4*P* and PtdIns4,5P_2. Under this condition, any enhanced turnover, even if accompanied by a decrease in mass, would lead to an increase in total radioactivity in PtdIns. When the loading was in a plasma-free solution (as for this study), however, the total radioactivity in phosphoinositides was 5–8-fold greater, and 16–29% of the phosphoinositide label was in PtdIns, indicating substantial labelling of the diester phosphate. After further incubation for 90 min (with or without Li^+), there was little change in total radioactivity in phosphoinositides or in the proportions of label in PtdIns:PtdIns4*P*:PtdIns4,5P_2 (approx. 1:1:1), which remained considerably higher than most published results (see comparisons by Tysnes *et al.*, 1985). (In contrast, when platelets were loaded in plasma, a subsequent incubation in a plasma-free solution for 90 min led to a substantial increase in label in PtdIns). Since our 90 min incubation after loading presumably also would lead to a lower specific radioactivity of ATP, a thrombin-induced increase in specific radioactivity of PtdIns would not occur; instead our results are more similar to those from measurements of mass (Broekman *et al.*, 1980; Bell & Majerus, 1980).

At 10 mM, Li^+ had no effect on the time courses of thrombin-induced changes in labelled PtdIns or PtdIns4,5P_2 (Fig. 4). There was, however, an effect on the response of labelled PtdIns4*P* to thrombin; instead of a slight increase in labelling, there was a statistically significant decrease, suggesting either enhanced hydrolysis to $InsP_2$ or $InsP_3$ (through PtdIns4,5P_2) or impaired resynthesis from PtdIns.

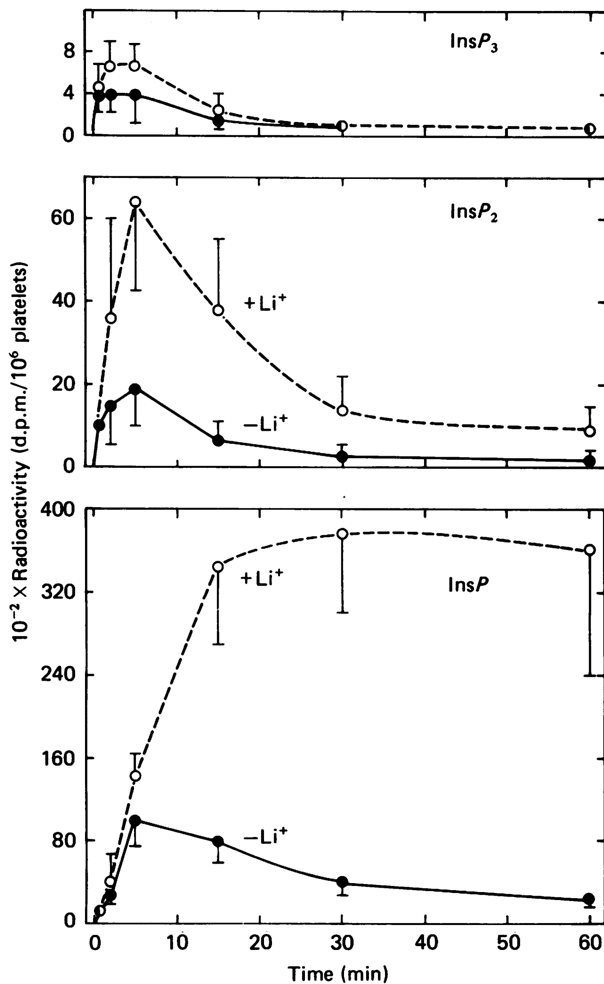

Fig. 3. Effects of Li^+ on extended time courses of thrombin-induced changes in $InsP_3$ $InsP_2$ and $InsP$ in control (●) and Li^+-treated (○) platelets

The experiment was as described for Figs. 1 and 2. After incubation with either 10 mM-LiCl or -NaCl for 90 min, platelets were activated with 15 nM-thrombin. The basal (before thrombin) radioactivity (d.p.m./10^6 platelets) in $InsP_3$, $InsP_2$ and $InsP$ was 216±124, 365±124 and 880±229 for control (NaCl) platelets and 211±109, 387±140 and ı ¨89±216 for Li^+-treated platelets.

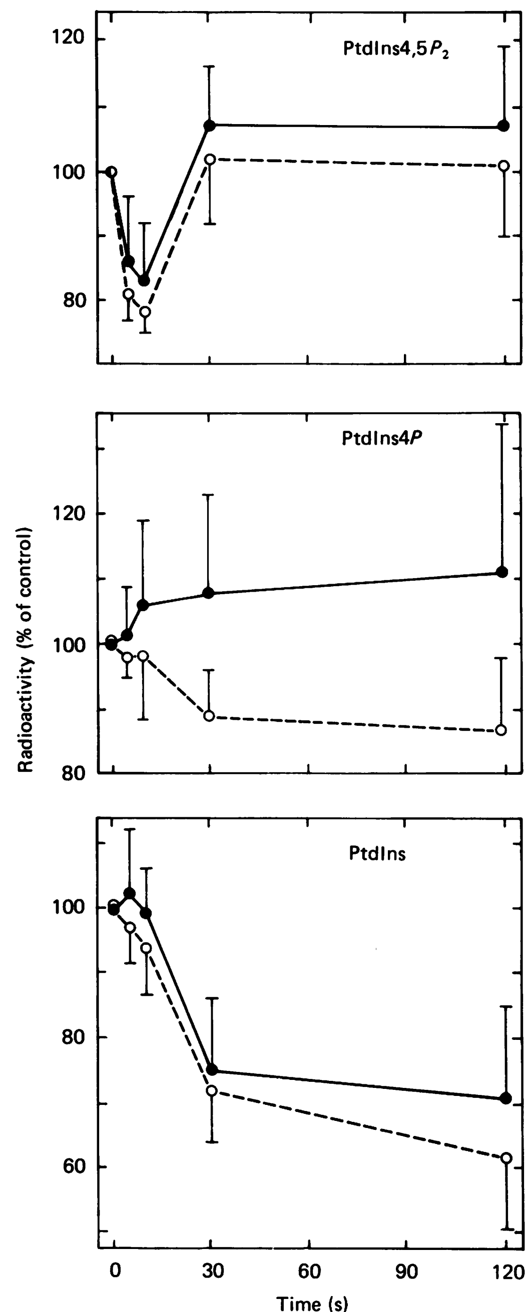

Fig. 4. Time courses of initial thrombin-induced changes in PtdIns4,5P, PtdIns4P and PtdIns in the absence (●) or presence (○) of 10 mM-LiCl

[^{32}P]Phosphate-labelled platelets were incubated at 37 °C for 90 min after addition of 10 mM-NaCl (control, ●) or 10 mM-LiCl (○). After addition of 15 nM-thrombin, labelled phospholipids were extracted and analysed at the indicated times as described in the Experimental section. The results (means ±S.E.M. for five separate experiments, each with triplicate measurements) are expressed as a percentage of the basal (before thrombin) radioactivity. The differences between control and Li^+-treated platelets at 30 and 120 s for PtdIns4P are statistically significant ($P < 0.05$). The basal values (c.p.m./10^6 platelets) for PtdIns4,5P_2, PtdIns4P and PtdIns were 21074±7612, 21351±7359 and 27088±9736 for control platelets and 21575±4298, 21699±4253 and 27119±7292 for Li^+-treated platelets.

Effect of Li^+ on the thrombin-induced production of [^{32}P]lyso-PtdIns

We also observed that Li^+ caused a greater thrombin-induced production of labelled lyso-PtdIns (Fig. 5), which presumably arises from the action of phospholipase A_2. This is consistent with the enhaיced thromboxane production in Li^+-treated platelets reported previously by Imandt *et al.* (1981),

DISCUSSION

We were interested in two reported effects of Li^+. It has been reported to enhance agonist-induced accumulation of inositol phosphates in some cells (Berridge *et al.*, 1982; Drummond *et al.*, 1984; Thomas *et al.*, 1984), and it has been reported to potentiate the response of human

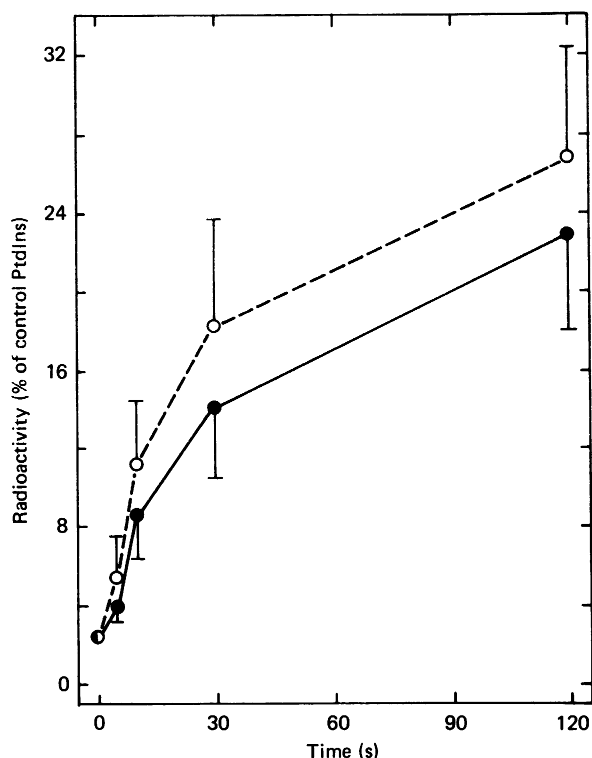

Fig. 5. Time courses of thrombin-induced increases in the amount of labelled lyso-PtdIns in control (●) and Li^+-treated (○) platelets

The experiment was as described for Fig. 4. The results (means ± S.E.M. for five separate experiments, each with triplicate measurements) are expressed as a percentage of the basal radioactivity in PtdIns. The basal radiolabelling (c.p.m./10^6 platelets) in lyso-PtdIns and PtdIns was 622 ± 195 and 21074 ± 7612 for control platelets and 616 ± 141 and 27119 ± 7292 for Li^+-treated platelets. The differences between control and Li^+-treated platelets at 30 and 120 s are statistically significant ($P < 0.05$).

platelets to agonists (Imandt *et al.*, 1977, 1980, 1981). If an inositol phosphate is a second messenger, the mechanism of potentiation by Li^+ might involve an increase in the concentration of the inositol phosphate either before the agonist or as a consequence of the agonist. We reasoned that potentiation by Li^+ could therefore involve a defined mechanism for studies of synergism and potentiation of platelet agonists. This is clearly not the case. Although inhibition of Ins*P* phosphatase is obvious, as predicted from published work with other cells, other effects on phosphoinositide metabolism are also apparent, and there is reason to question whether effects on phosphoinositide metabolism are in fact the mechanism of potentiation by Li^+.

We chose to analyse phosphoinositide metabolites by incorporation of radioactive precursors, because direct measurements of mass require large amounts of very concentrated platelet suspensions and do not reveal changes in small, metabolically active, pools or changes in rates of turnover. Measurements of radioactivity are sufficiently sensitive, but they require cautious interpretation. It is unlikely that platelets have a homogeneous pool of phosphoinositides, and an increased rate of turnover of a metabolite will result in either an increase or a decrease in radioactivity, depending on whether the precursor has a higher or lower specific radioactivity. We therefore restrict our interpretation to comparisons between control and Li^+-treated platelets.

The inhibition by Li^+ of Ins*P* phosphatase in intact human platelets is apparent from the large thrombin-induced accumulation of labelled Ins*P* without a subsequent decline after other labelled inositol phosphates had returned to near-basal radioactivity values (Fig. 3). Other effects of Li^+ on thrombin-activated phosphoinositide metabolism were apparent as an enhanced accumulation of labelled InsP_2 and InsP_3 (Figs. 2 and 3), as a thrombin-induced decrease in labelled PtdIns4*P* (Fig. 4) and as an enhanced thrombin-induced accumulation of labelled lyso-PtdIns (Fig. 5). Although accumulation of labelled InsP_2 and InsP_3 could indicate inhibition of their phosphatases, it could be only a slight inhibition, because return to basal values occurred at the same rates (half-times) in control and Li^+-treated platelets. Other explanations are also plausible. For example, the observation that Li^+ caused a thrombin-induced depletion of labelled PtdIns4*P* indicates that at least part of the enhanced accumulation of labelled InsP_2 could have been due to more rapid production, consistent with the observation by Drummond & Raeburn (1984) that Li^+ caused enhanced agonist-induced production of diacylglycerol in GH_3 pituitary cells. Our measurements of diacylglycerol accumulation in thrombin-activated platelets were, unfortunately, inconclusive, and we cannot estimate the extent to which an increased rate of hydrolysis could account for the greater, transient, accumulation of labelled InsP_2. The enhanced production of labelled lyso-PtdIns indicates that Li^+ also had some effect unrelated to either production or degradation of inositol phosphates.

In similar experiments with rabbit platelets, Vickers *et al.* (1984) observed a 3–6-fold greater accumulation of labelled InsP_3 30–120 s after addition of thrombin, with a 2-fold greater accumulation of labelled InsP_2 at the 120 s time point only. In contrast with our observations, they found Li^+ effects at the earliest time points after addition of the agonist, and they found no effect consistent with inhibition of Ins*P* phosphatase. The failure to see inhibition of Ins*P* phosphatase was probably because they measured intermediates for only 2 min after addition of thrombin, whereas the effect of Li^+ is not apparent until later (Fig. 3). Watson *et al.* (1984) observed little effect of Li^+ on [^{3}H]inositol-labelled InsP_2 or InsP_3 for 2 min after addition of thrombin to washed human platelets, but their observed effect of Li^+ on the thrombin-induced increase in Ins*P* was greater than we report here. Since their data for control and Li^+-treated platelets were from separate experiments, exact quantitative comparisons are, however, difficult. Siess (1985) reported that incubation of human platelets with Li^+ caused an increase in labelled Ins4*P* as well as Ins1*P*; Li^+ enhanced the thrombin-induced accumulation of label in Ins1*P*, and the vasopressin-induced increases in both Ins1*P* and Ins4*P*.

We conclude that our data, together with published data for other cell systems, indicate that in intact human platelets Li^+ inhibits Ins*P* phosphatase, but that there are, in addition, other effects on thrombin-induced phosphoinositide metabolism. It is impossible to deduce from these data which were primary and which were secondary effects of Li^+.

Some of the effects of Li^+ that we report here have certain characteristics essential for an event that causes potentiation. It is apparent that the enhanced thrombin-induced accumulation of labelled inositol phosphates in Li^+-treated platelets was not simply a reflection of greater sensitivity to thrombin, because the effect was a qualitative change rather than a shift in a dose–response curve (Fig. 2). Thus an enhanced accumulation of a postulated second messenger appears to be a primary effect. It does not, however, appear to be the cause of potentiation. Since the extent of thrombin-induced platelet activation is determined quickly, any cause of an enhanced response to thrombin should also occur quickly, but the effect of Li^+ on thrombin-induced phosphoinositide metabolism was not apparent until 30 s after addition of thrombin. It may, however, be significant that the potentiation by Li^+ of platelet aggregation and secretion that we observe tends to be greater at later times ($>$ 30 s), consistent with most of the potentiation described by Imandt *et al.* (1977, 1980).

A major difficulty in assessing the possible role of phosphoinisotide metabolism in potentiation by Li^+ is that there appears to be no simple correlation of amount of postulated second messenger with extent of response (see, e.g., Thomas *et al.*, 1984). Thus, if InsP_3 is a second messenger, the effect of Li^+ on its accumulation in response to thrombin would be comparable to at least doubling the concentration of thrombin, but such a great effect on platelet responses is never observed. This may indicate that the effect on phosphoinositide metabolism is not the mechanism of potentiation, or it may only reveal a lack of direct proportionality of concentration of InsP_3 and extent of activation, perhaps because only one of the measured InsP_3 isomers is active (Irvine *et al.*, 1984), as suggested by Burgess *et al.* (1985).

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REFERENCES

Allison, J. H. & Stewart, M. A. (1971) Nature (London) New Biol. **233**, 267–268
Allison, J. M., Blisner, M. E., Holland, W. H., Hipps, P. P. & Sherman, W. R. (1976) Biochem. Biophys. Res. Commun. **71**, 664–670
Aster, R. H. & Jandl, J. H. (1964) J. Clin. Invest. **43**, 843–856
Bell, R. L. & Majerus, P. W. (1980) J. Biol. Chem. **255**, 1790–1792
Berridge, M. J. (1984) Biochem. J. **220**, 345–360
Berridge, M. J., Downes, C. P. & Hanley, M. R. (1982) Biochem. J. **206**, 587–595
Billah, M. M. & Lapetina, E. G. (1982) J. Biol. Chem. **257**, 12705–12708
Broekman, M. J., Ward, J. W. & Marcus, A. J. (1980) J. Clin. Invest. **66**, 275–283
Burgess, G. M., McKinney, J. S., Irvine, R. F. & Putney, J. W., Jr. (1985) Biochem. J. **232**, 237–243
Connolly, T. M., Bross, T. E. & Majerus, P. W. (1985) J. Biol. Chem. **260**, 7868–7874
Downes, C. P. & Michell, R. H. (1981) Biochem. J. **198**, 133–140
Drummond, A. H. & Raeburn, C. A. (1984) Biochem. J. **224**, 129–136
Drummond, A. H., Bushfield, M. & Macphee, C. H. (1984) Mol. Pharmacol. **25**, 201–208
Hallcher, L. M. & Sherman, W. R. (1980) J. Biol. Chem. **255**, 10896–10901
Holmsen, H., Dangelmaier, C. A. & Holmsen, H. K. (1981) J. Biol. Chem. **256**, 9393–9396
Holmsen, H., Dangelmaier, C. A. & Rongved, S. (1984) Biochem. J. **222**, 157–167
Imandt, L., Genders, T., Wessels, H. & Haanen, C. (1977) Thromb. Res. **11**, 297–308
Imandt, L., Tijhuis, D., Wessels, H. & Haanen, C. (1980) Haemostasis **9**, 176–187
Imandt, L., Tijhuis, D., Wessels, H. & Haanen, C. (1981) Prostaglandins Med. **7**, 421–429
Irvine, R. F., Letcher, A. J., Lander, D. J. & Downes, C. P. (1984) Biochem. J. **223**, 237–243
Nishizuka, Y. (1984) Science **225**, 1365–1370
Rittenhouse, S. E. & Sasson, J. P. (1985) J. Biol. Chem. **260**, 8657–8660
Seyfred, M. A., Farrell, L. E. & Wells, W. W. (1984) J. Biol. Chem. **259**, 13204–13208
Sherman, W. R., Leavitt, A. L., Honchar, M. P., Hallcher, L. M. & Phillips, B. E. (1981) J. Neurochem. **36**, 1947–1951
Siess, W. (1985) FEBS Lett. **185**, 151–156
Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. (1983) Nature (London) **306**, 67–69
Takai, Y., Kishimoto, A., Kikkawa, U., Mori, T. & Nishizuka, Y. (1979) Biochem. Biophys. Res. Commun. **91**, 1218–1224
Thomas, A. P., Alexander, J. & Williamson, J. R. (1984) J. Biol. Chem. **259**, 5575–5584
Tysnes, O.-B., Aarbakke, G. M., Verhoeven, A. J. M. & Holmsen, H. (1985) Thromb. Res. **40**, 329–338
Vickers, J. D., Kinlough-Rathbone, R. L. & Mustard, J. F. (1984) Biochem. J. **224**, 399–405
Watson, S. P., McConnell, R. T. & Lapetina, E. G. (1984) J. Biol. Chem. **259**, 13199–13203

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