Cationic factors affecting phospholipase activities from human lung

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- 1 The effects of varying H⁺ and other cation concentrations on phospholipase activity were investigated on two particulate fractions from human lung, corresponding to the mitochondrial and microsomal fractions.
- 2 Three ¹⁴C-labelled substrates, arachidonyl-phosphatidylcholine (PC), -phosphatidylethanolamine (PE) and -phosphatidylinositol (PI) were used.
- 3 For two substrates, PE and PI, hydrolysis was maximal at pH 6, with either subcellular fraction.
- 4 Hydrolysis of all three substrates was strongly inhibited by EDTA and EGTA (10–25 mM). Addition of 2,2-dipyridyl, *o*-phenanthroline, 8-hydroxyquinoline or desferrioxamine (10 μ M–1 mM) did not inhibit but often increased hydrolysis of all substrates.
- 5 Addition of Zn²⁺, as ZnCl₂, (10 μ M–1 mM) inhibited PE and PI, but not PC, hydrolysis.
- 6 The phospholipase activities from human lung appear to be dependent on Ca²⁺ for maximal activity and to be inhibited by other metal ions including Zn²⁺.

Introduction

Phospholipases liberating arachidonate from endogenous lipids play a crucial role in the overall control of eicosanoid biosynthesis (Flower & Blackwell, 1976). The eicosanoids exhibit a wide range of potent biological activities on a variety of cells in the lung and these activities are expressed in both physiological and pathological conditions (Bakhle & Ferreira, 1985). Phospholipase action has also been linked to receptor activation by many different agonists effective in lung (Fain *et al.*, 1988). Thus phospholipases are involved crucially in both inter- and intracellular signalling in lung and a fuller knowledge of lung phospholipases is essential to our understanding of pulmonary physiology and pathophysiology in man.

We have shown the presence of a phospholipase A₂ (PLA₂)-like activity and of a PLC-like activity in cell-free extracts of human lung (Bakhle & Yeats, 1986; Yeats & Bakhle, 1989b). To characterize further these activities exhibited towards phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI), we have investigated the effects of pH and of Ca²⁺ and Zn²⁺ ions on the hydrolysis of these substrates, labelled with [¹⁴C]-arachidonic acid in the sn-2 position. A preliminary account of some of the results have been communicated to the British Pharmacological Society (Bakhle & Yeats, 1988; Yeats & Bakhle, 1989a).

Methods

Preparation of subcellular fractions of human lung homogenate

Samples of human lung obtained at surgery and macroscopically normal were chopped with scissors, rinsed with cold (4°C), sterile isotonic saline and homogenized in 4 volumes of cold standard buffer with 6 × 10 s bursts of a Polytron blade homogenizer. The standard buffer used throughout this work contained (mM): Tris HCl (pH 7.4) 10, sucrose 250, CaCl₂ 5. All processing of the lung extracts was carried out at 4°C.

Subcellular fractions were derived from the homogenate by differential centrifugation as described earlier (Yeats & Bakhle, 1989b). In this set of experiments, only the particulate

fractions P₁ and P₂ were used. Mitochondrial and lysosomal enzymes were concentrated in the P₁ fraction and microsomal enzymes in the P₂ fraction (Yeats & Bakhle, 1989b). These fractions exhibited higher specific activity than the supernatant fraction (S₂ obtained after 100 000 *g* for 60 min): P₂:S₂ activity ratios were 15 for PC, 13 for PE and 360 for PI. Further details of the distribution of phospholipase activity in the various subcellular fractions have already been given (Yeats & Bakhle, 1989b).

Phospholipase assay

This assay has been fully described elsewhere (Yeats & Bakhle, 1989b). The standard incubation mixture (100 μ l) contained about 2 × 10⁵ d.p.m. of [¹⁴C]-arachidonyl-PC, PE or PI (15 μ M); 5 mM CaCl₂; 10 mM Tris HCl, pH 7.4 and various amounts of lung protein. The amount of protein depended on the substrate; with PC, 20–30 μ g, and with PE and PI, 10–20 μ g were used. Hydrolysis of each substrate was linearly proportional to the amount of protein used from 3–30 μ g per incubation sample (data not shown). After 40 min incubation at 37°C, the reaction was stopped with 25 μ l of 2 M HCl and 10 μ l of 0.6 mM arachidonic acid (AA) added. Then 1 ml of the first eluting solvent (1a or 1b, see below) was added and the two phases mixed vigorously before application to the silicic acid columns for analysis.

Non-enzymic hydrolysis of phospholipid substrates was measured by incubation with buffer alone and these values subtracted from those derived from incubation with lung fractions. Where necessary the buffer contained the additional cations, chelating agents etc., under study. These additions were made up in the standard buffer to provide the required final concentrations in the incubation mixture.

Analysis of hydrolysates

Silicic acid column chromatography Glass Pasteur pipettes (145 × 5 mm) were plugged with glass wool and a slurry of silicic acid in eluting solvent 1a (hexane:dioxane:glacial acetic acid, 80:20:1, v/v) poured in to form a column 3 cm high. To these columns, the emulsified reaction mixture was applied and hydrolysis products eluted with a further 5 ml of solvent 1a. Another solvent, solvent 2, (CHCl₃:MeOH:H₂O, 65:35:1, v/v) was used to elute unchanged substrate.

This two solvent system was not adequate for the full analysis of PI-derived hydrolysates which contained labelled

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diglyceride (DG) as well as AA and both these products were eluted together by solvent 1a. Thus for incubations with PI, another less polar solvent, solvent 1b (hexane:dioxane:glacial acetic acid, 97:2:1, v/v) was devised and used both to prepare the columns and as the first eluting solvent, before solvent 1a. All three labelled species, AA, DG, and PI, were very effectively separated by these three solvents. From incubation samples, the radioactivity in eluate 1 (solvent 1b) was chiefly associated with marker AA (98%), that in eluate 2 (solvent 1a) with DG (93%) and in eluate 3 (solvent 2) with PI (96%), on analysis by thin layer chromatography (t.l.c., see below). Further metabolism of the product AA to prostaglandins was negligible; marker prostaglandin E_2 (PGE_2) or 6-oxo- $PGF_{1\alpha}$ was eluted with solvent 2 and less than 2% of ^{14}C eluted with solvent 2 from incubation mixtures co-chromatographed with these prostaglandins on subsequent t.l.c. Routinely, 6 ml of each solvent gave good recovery of the radioactivity in the incubation mixture applied to the column (>85% for product and >80% for substrate). The total eluate of each solvent was combined to provide a single fraction for radioactivity measurements.

Thin layer chromatography Each eluate from the column was analysed by t.l.c. in which silica gel layers on aluminium foil were used. The eluates were evaporated under reduced pressure and the residues redissolved in $CHCl_3$:MeOH (2:1, v/v, 200 μ l) and applied to the t.l.c. plates with a mixture of marker compounds. Development was with two solvent systems depending on the possible contents of the eluate; to separate prostaglandin and substrate, we used $CHCl_3$:MeOH; glacial acetic acid, 90:5:5, v/v (Flower & Blackwell, 1976) and to separate AA and glycerides and prostaglandins, we used hexane:diethyl ether:glacial acetic acid, 50:50:1, v/v (Jose & Seale, 1979). After development the plates were dried, markers visualized with iodine vapour and each sample track cut into 1 cm strips. These were placed in scintillation vials with 2 ml MeOH and, after 2 h at room temperature, 8 ml of scintillant fluid added and the radioactivity measured.

Measurement of radioactivity

All radioactive samples were assayed by liquid scintillation methods with external standards used to assess quenching. Radioactivity was expressed as d.p.m.

Other assays

Protein in the lung fractions was estimated by the method of Bradford (1976).

Variation of activity with pH

Incubations at pH 4 and 5 used sodium acetate buffers; at pH 6, Tris maleate and at other pH values, Tris HCl buffers were used, all 10 mM. All pH values were measured at 37°C.

Materials

We are most grateful to Mr J. Dussek, F.R.C.S., Guy's Hospital, London for samples of human lung. The following radioactive compounds were obtained from Amersham International, Bucks.: PC, 1-stearoyl-2-[1- ^{14}C]arachidonyl-3-phosphatidylcholine; PE, 1-acyl-2-[1- ^{14}C]arachidonyl-3-phosphatidylethanolamine; PI, 1-stearoyl-2-[1- ^{14}C]arachidonyl-3-phosphatidylinositol, all 58–60 mCi mmol $^{-1}$. Aqueous liquid scintillation cocktail, 'Optiphase Safe', was obtained from LKB (Loughborough). Other materials were obtained as follows; Tris base and marker compounds for t.l.c. from Sigma (Poole); silicic acid (Bio-Sil A), 100–200 mesh (Bio-Rad Laboratories, California, USA); t.l.c. plates, silica gel 60 on aluminium foil (Merck, Darmstadt, F.R.G.). All other chemi-

cals were of Analar grade and supplied by BDH Chemicals (Poole).

Statistical methods

Results given are the mean values from at least six assays from at least three lungs. The paired *t* test was used to assess the significance of the difference between means and a value of $P < 0.05$ was taken to denote a significant difference.

Results

Analysis of phospholipid hydrolysis

Under the original conditions (pH 7.4 and 5 mM Ca^{2+}), PC and PE were hydrolysed to a single radioactive product, free AA, but PI yielded two labelled metabolites, diglyceride (DG) and, as the lesser component, free AA (Yeats & Bakhle, 1989b). Since variations in pH and other cations might affect these products differently, each product had to be measured separately. For this reason we modified the column chromatography, for PI only, to yield separate eluates for each species; AA, DG and unchanged PI. Because each lung sample was assayed separately, the effects of variations of pH, Ca^{2+} or other ionic factors on phospholipase activity were related to hydrolysis under the original conditions and expressed as a percentage of that value, referred to henceforward as 'control hydrolysis'. The mean ($\pm$ s.e.mean) phospholipase activity towards PC was 15 ± 2 ($n = 5$ lungs), towards PE, 203 ± 23 ($n = 6$) and for PI, 907 ± 179 ($n = 6$) pmol min $^{-1}$ mg $^{-1}$ protein.

Variation of phospholipid hydrolysis with pH

Because hydrolysis of PC proceeded relatively slowly under our control conditions (about 7% of the rate of PE hydrolysis (see above)), we used only PE as the substrate for PLA $_2$ -like activity. Over the range of pH studied (pH 4–9), PE hydrolysis was fairly constant, the maximum activity ratio being about 3 between the lowest activity at pH 4, about 50% of the control value, and at pH 6, about 150% of control (Figure 1). At the highest pH studied (pH 9), hydrolysis was only slightly reduced from the control value. This pH profile was virtually the same for the two particulate fractions (P_2 , microsomal and P_1 , mitochondrial and lysosomal).

Hydrolysis of PI was first measured as total hydrolysis, i.e. DG and AA together. Although fractions P_1 and P_2 again showed similar profiles (Figure 2), the magnitudes of the pH-induced changes were greater than with PE. A further difference was that activity towards PI was retained at pH values below 6. The maximum hydrolysis at pH 6 was almost three times control with the P_2 fraction and the minimum at pH 9 was about half the control value for both P_1 and P_2 fractions.

Figure 1 Variation of phosphatidylethanolamine (PE) hydrolysis with pH. The values shown are the mean hydrolysis (with s.e.mean shown by vertical bars), expressed as a percentage of the hydrolysis under control conditions at pH 7.4. Activity of both P_1 (●) and P_2 (○) fractions was almost identically affected by pH with a peak at pH 6 and a minimum value at pH 4.

Figure 2 Variation of phosphatidylinositol (PI) hydrolysis by particulate fractions of human lung with pH. The values shown are the mean total hydrolysis, (with s.e.mean shown by vertical bars), measured as arachidonic acid plus diglyceride produced, expressed as a percentage of the total hydrolysis under control conditions at pH 7.4. Activity of both P_1 (●) and P_2 (○) fractions was highest at pH 6 and lowest at pH 9.

Further analysis of PI hydrolysis into AA and DG production separately showed that DG production remained higher than that of AA at pH 6 and that it fell more steeply at high pH, as shown for fraction P_2 in Figure 3. As a result of this disparate response to pH, the AA:DG ratio increased at pH 9, from 0.3 to 0.6 with the P_1 fraction and from 0.2 to 0.3 with the P_2 fraction.

Effects of other cations on phospholipase activity

The control incubation mixture included Ca^{2+} (5 mM; final concentration) as this ion is known to be required by several phospholipases including those derived from lung (van den Bosch, 1982). Increasing the Ca^{2+} concentration to 9.5 mM increased hydrolysis of PC by the P_2 fraction to $140 \pm 15\%$ (mean $\pm$ s.e.mean) of the control value but did not change PE hydrolysis ($89 \pm 10\%$). For PI, while DG production was maintained ($103 \pm 3\%$), that of AA fell to $75 \pm 9\%$. However because AA comprised only about 15% of the total hydrolysis products, overall hydrolysis was unchanged ($99 \pm 5\%$).

Lowering Ca^{2+} concentrations by the addition of EDTA and EGTA inhibited phospholipase activity markedly (Table 1) but, even with a considerable excess of EDTA (25 mM), the activity was never abolished. This general pattern of response to EDTA and EGTA was shown by the activities in both sub-cellular fractions. However, with fraction P_1 , hydrolysis of PC and PE were equally inhibited by these chelating agents but, with P_2 , the microsomal fraction, PE hydrolysis was more sensitive to inhibition. The PLC activity was also inhibited by

Figure 3 Variation of phosphatidylinositol (PI) hydrolysis by the P_2 (microsomal) fraction of human lung with pH. The values shown are the mean values (with s.e.mean shown by vertical bars) of arachidonic acid (AA, ○) and diglyceride (DG, ●) produced, measured separately. They are expressed as a percentage of the production under control conditions at pH 7.4. Production of DG was well above control levels at low pH but fell more sharply than that of AA at high pH.

Table 1 Phospholipid hydrolysis by particulate fractions of human lung in the presence of EDTA and EGTA

	Hydrolysis of substrate (% control value)		
	PC	PE	PI
Fraction P_1 (mitochondrial and lysosomal)			
Na EDTA; 25 mM	20 $\pm$ 4	20 $\pm$ 5	—
Na EGTA; 10 mM	38 $\pm$ 8	33 $\pm$ 8	—
Fraction P_2 (microsomal)			
Na EDTA; 25 mM	38 $\pm$ 11	8 $\pm$ 2	11 $\pm$ 3
Na EGTA; 10 mM	42 $\pm$ 11	18 $\pm$ 4	—

The values in the Table are the mean ($\pm$ s.e.mean) results expressed as a percentage of the corresponding control hydrolysis, from at least 6 assays from 3 lungs at each point. All the conditions yielded significant inhibition of hydrolysis. PC phosphatidylcholine; PE phosphatidylethanolamine; PI phosphatidylinositol.

EDTA, total PI hydrolysis falling to about 10% of normal values. Production of the DG component was more affected, resulting in an increase in the AA/DG ratio to 0.5 in the presence of EDTA, over the control value of 0.2.

Further analysis of the cation dependence of the phospholipase activities, in the P_2 fraction only, was attempted with the calcium salt of EDTA (CaEDTA) and other chelating agents (Table 2). In the presence of CaEDTA (5 mM), PC and PE hydrolysis by the microsomal fraction was strongly inhibited, but that of PI was increased by about 30%, with no change in AA:DG ratio.

Table 2 Effects of chelating agents on phospholipase activity in the P_2 (microsomal) fraction of human lung

Chelating agent (concentration)	Hydrolysis of substrate (% control value)		
	PC	PE	PI
Ca EDTA (5 mM)	30 $\pm$ 5	15 $\pm$ 3	135 $\pm$ 10
<i>o</i> -Phenanthroline			
0.1 mM	159 $\pm$ 26	276 $\pm$ 23	177 $\pm$ 31
1 mM	214 $\pm$ 35	214 $\pm$ 25	219 $\pm$ 19
8-Hydroxyquinoline			
0.1 mM	—	139 $\pm$ 25	126 $\pm$ 12
1 mM	281 $\pm$ 27	182 $\pm$ 40	196 $\pm$ 37
2,2-Dipyridyl			
0.1 mM	114 $\pm$ 13 (NS)	200 $\pm$ 12	132 $\pm$ 18
1 mM	220 $\pm$ 39	177 $\pm$ 19	202 $\pm$ 28
Desferrioxamine			
1 mM	—	106 $\pm$ 10 (NS)	108 $\pm$ 2

The values in the Table are the mean ($\pm$ s.e.mean) results expressed as a percentage of the corresponding control hydrolysis, from at least 6 assays from 3 lungs at each point. All the effects were significant except those marked (NS). Inhibition was only seen with CaEDTA and phosphatidylcholine (PC) and phosphatidylethanolamine (PE) hydrolysis; all of the other effects were stimulatory. PI phosphatidylinositol.

With the four other chelating agents used, a completely different profile emerged (Table 2). For any substrate with any concentration of chelator, hydrolysis catalysed by the microsomal fraction was never inhibited. Two agents, *o*-phenanthroline and 2,2-dipyridyl, were tested at a lower concentration (10 μ M) with PC as substrate and were without effect (117 $\pm$ 14% and 90 $\pm$ 12% of control values respectively). Furthermore, at the higher concentration of chelator (1 mM), hydrolysis was always increased over normal values. There were minor variations within this group of chelating agents; *o*-phenanthroline was effective at 100 μ M with all substrates, whereas 8-hydroxyquinoline was effective only at 1 mM. Desferrioxamine was the least effective, increasing PI hydrolysis only slightly at 1 mM. The AA:DG ratio of products of PI hydrolysis was not changed by any of this group of agents.

The effects of these four chelating agents were in marked contrast to those of EDTA and EGTA. However, the former group was used in concentrations only up to 1 mM and the latter two not below 10 mM. It was possible that the discrepant results were due to the different concentration ranges used.

To eliminate this possibility, hydrolysis of PE and PI was measured at low EDTA (1 mM) and high 2,2-dipyridyl (25 mM) concentrations. PE hydrolysis was not changed by either treatment, 99 $\pm$ 8% of the control value with 1 mM EDTA and 88 $\pm$ 10% with 2,2-dipyridyl. However, PI hydrolysis was increased by 1 mM EDTA (133 $\pm$ 5%) and by 2,2-dipyridyl (122 $\pm$ 10%) with no change in the AA:DG ratio.

In the light of these results and the report of inhibition of purified PLA₂ by Zn²⁺ ions (Finnen *et al.*, 1986), we investigated the effects of ZnCl₂ added to the incubation mixture. Using the P₂ fraction, hydrolysis of PC was measured at 9.5 mM Ca²⁺, the optimal concentration for this substrate but PE and PI hydrolysis were measured at 5 mM Ca²⁺ as their hydrolysis was not affected by increased Ca²⁺. As shown in Table 3, addition of ZnCl₂ to a final concentration of 100 μ M slightly decreased PC hydrolysis but, at the higher concentrations, PC hydrolysis was equal to normal values. However, for PE and PI hydrolysis, the inhibition was more extensive and concentration-related. The AA:DG ratio in PI hydrolysates was clearly affected by Zn²⁺ from a control value of 0.45 (no added Zn²⁺) to 0.77 at 100 μ M and to 1.13 at 1 mM Zn²⁺, demonstrating a relatively greater inhibition of DG than of AA production.

Discussion

In this series of experiments, we have examined the effects of some cations (including H⁺) on the phospholipase activities in human lung extracts using, for the most part, the microsomal fraction (P₂). Because our lung samples derived from a genetically wild population, we did not combine samples to give a single 'average' preparation but kept each sample separate. All the assays utilized at least three different lung samples but the three lungs were not the same throughout these experiments. However, within a group of assays, e.g. pH variations, Ca²⁺ or Zn²⁺ variations, the same lung samples were used.

pH dependence

The dependence of PLA₂ activity on pH was assayed only with PE as substrate as the activity towards PC was much lower (see Figure 1 and Table 1). The results demonstrated a pH optimum at about pH 5–6, with relatively higher activity at high pH than at low pH. Both the particulate fractions tested showed a similar profile.

Our results contrast with most of the previous work on membrane-bound PLA₂. Non-lysosomal PLA₂ activities from human neutrophils (Balsinde *et al.*, 1988), rat platelets (Colard *et al.*, 1987; Blache & Ciavatti, 1987) and liver (Victoria *et al.*, 1971; Nachbaur *et al.*, 1972; Newkirk & Waite, 1973) or from polymorphonuclear leukocytes (PMNs) of rat, rabbit and man

Table 3 Effect of added Zn²⁺ on phospholipase activity of P₂ (microsomal) fraction from human lung

Concentration of ZnCl ₂	Hydrolysis of substrate (% control value)		
	PC	PE	PI
10 μ M	—	43 $\pm$ 3*	73 $\pm$ 9*
100 μ M	85 $\pm$ 5*	28 $\pm$ 3*	38 $\pm$ 7*
1 mM	98 $\pm$ 9	28 $\pm$ 3*	26 $\pm$ 3*

* Different from control value i.e., no added ZnCl₂ (= 100%).

The values in the Table are the mean ($\pm$ s.e.mean) results expressed as a percentage of the corresponding control hydrolysis, from at least 6 assays from 3 lungs at each point. Hydrolysis of phosphatidylcholine (PC) was much less affected by Zn²⁺ than that of either phosphatidylethanolamine (PE) or phosphatidylinositol (PI).

(Lanni & Becker, 1983; Chang *et al.*, 1985; Alonso *et al.*, 1986), all have pH optima between pH 7.5 and pH 9. Lysosomal PLA₂ from human liver (Löffler & Kunze, 1987) or from rabbit alveolar macrophages (Franson *et al.*, 1973) shows an acid optimum at about pH 4. Although we were able to show a relative concentration of lysosomal marker enzymes in the P₁ fraction, our results on PLA₂ activity in P₁ showed no sign of a pH optimum markedly different from that for the PLA₂ activity in the microsomal fraction (P₂). A closer analogy might be sought in non-human lung PLA₂ but, here also, the membrane-bound enzymes are clearly of neutral-basic pH optima, values ranging between pH 7.5 and pH 9.5 for rat, rabbit and dog lung (Garcia *et al.*, 1975; Longmore *et al.*, 1979; Franson & Weir, 1982; Scott *et al.*, 1987).

Our subcellular fractions may contain a number of PLA₂ enzymes with either acid or alkaline optima which might in combination yield the profiles observed. However, such a mixture might reasonably be different in mitochondrial and microsomal fractions and thus give rise to different pH-activity profiles, in contrast to our results. Another possible explanation is that, because our lung samples derived from patients, i.e. with some pathological condition, our results represent the phospholipase activities in lung responding to a pathological insult, whereas most previous studies have used normal animals or man. However, because other properties of these human lung PLA₂ and PLC activities are similar to those of normal rat lung (Yeats & Bakhle, 1989b), we consider that our samples of human lung probably represent normal tissue and, by extension, the properties of the phospholipases should also reflect that of the normal enzymes. The pH dependence of PI hydrolysis in our lung extracts was more marked with the P₂ fraction, although both fractions exhibited maximum activity at pH 6. A lysosomal PI-specific PLC from rat liver had a pH optimum of 4.8 with very little activity above pH 7.0 (Irvine *et al.*, 1978). Our findings are closer to the soluble PLCs from heart (Low & Weglicki, 1983) and lymphocytes (Carter & Smith, 1987) with maxima at pH 5.5–6.0. In the lymphocyte preparation before purification, there was a minor component with activity at pH 7.0 which may represent a membrane associated form of this PLC (Carter & Smith, 1987). However, our results could as easily reflect a mixture of PLCs with acid and acid-neutral pH optima.

Effects of Ca²⁺ and other cations

In common with many other phospholipase activities, the presence of Ca²⁺ appeared to be essential for maximal activity in human lung extracts. The increased PC but not PE hydrolysis at higher Ca²⁺ concentrations could indicate either different enzymes for each substrate or different binding requirements for the same enzyme. A full examination of Ca²⁺-dependence is needed for both PLA₂ and PLC activities in these fractions. Drastic reductions in Ca²⁺ with excess EDTA or EGTA lead

to equally drastic reductions in activity towards all three substrates. However the relative resistance to EDTA of P_1 catalysed hydrolysis of PE and of PC hydrolysis by both P_1 and P_2 fractions would be compatible with the presence of a lysosomal, Ca^{2+} -independent PLA_2 activity (Franson *et al.*, 1973).

We attempted to explore the requirement for other cations by using other chelating agents, with greater affinity for transition metal ions. The potentiation of hydrolysis suggests the removal of an inhibitory factor which, on the basis of previous findings (Wolf & Gross, 1985; Finnen *et al.*, 1986) and our own results, could be Zn^{2+} ions. However, 8-hydroxyquinoline, 2,2-dipyridyl and *o*-phenanthroline all potentiated PC hydrolysis, but this hydrolysis was not inhibited by added Zn^{2+} ions. This could be because there were already enough Zn^{2+} ions present in the P_2 fraction to inhibit PC-hydrolysis maximally and addition of further Zn^{2+} ions was thus ineffective. Nevertheless, removal of all Zn^{2+} ions by chelation would still increase PC-hydrolysis. As a corollary, PE and PI hydrolysis would require more Zn^{2+} ions than present in P_2 for maximal inhibition and would thus appear to be more sensitive to added Zn^{2+} . Such an explanation would also be compatible with the lower PC hydrolysis in all lung fractions and with the higher concentrations of 2,2-dipyridyl and *o*-phenanthroline needed to potentiate PC hydrolysis. From our experiments, it is not possible to decide between an interaction with enzyme or with substrate as the mechanism for inhibition by Zn^{2+} , but Finnen *et al.* (1986) favour the former.

In summary, our experiments with human lung phos-

pholipases have shown that phospholipid hydrolysis was maximal at about pH 6. This profile is unlike that of many other PLA_2 activities and may be an intrinsic property of human lung PLA_2 s or, possibly, a reflection of pathology. These human lung phospholipases were also dependent on Ca^{2+} and their activity was modified by other cations, including Zn^{2+} . This last finding suggests an additional cationic control mechanism for the generation of arachidonate and diglyceride in lung and perhaps in other tissues. Our results provide more information on the properties of the phospholipases in human lung involved in liberating arachidonate and thus controlling eicosanoid biosynthesis in human lung.

These results have at least two important pharmacological corollaries. First, we can now measure the effect of activators and inhibitors of phospholipases (peptides, cytokines, lipocortins) directly on human lung enzymes without having to infer their effects from experiments with animal tissues. Second, where animal models still must be used, we can now select those species whose phospholipase activities most clearly resemble those in human lung. There are important species differences in the biosynthesis of cyclo-oxygenase products in lung (Bakhle & Ferreira, 1985) and these differences could extend to the phospholipases involved. Both considerations will allow a more accurate analysis of how human lung responds to pathological conditions such as endotoxin shock or immunological challenge and to drug treatments aimed at preventing such responses.

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