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The inhibition of diacylglycerol-stimulated intracellular phospholipases by phospholipids with a phosphocholine-containing polar group

A possible physiological role for sphingomyelin

Rex M. C. DAWSON, Norma HEMINGTON and Robin F. IRVINE
Department of Biochemistry, Agricultural and Food Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.

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1. Phosphatidylinositol phosphodiesterase activated by diacylglycerol is substantially inhibited by all phospholipids containing a phosphocholine head group, including phosphatidylcholine, hydrogenated phosphatidylcholine, choline plasmalogen, lysophosphatidylcholine, lysocholine plasmalogen, sphingomyelin and sphingosylphosphocholine. The sphingosine-containing phospholipids are the most inhibitory. Phosphatidic acid does not inhibit, and phosphatidylethanolamine activates the hydrolysis still further. 2. Sphingomyelin is highly inhibitory to a diacylglycerol-stimulated intestinal mucosal phospholipase A_2, or a liver lysosomal phospholipase $A_1 + A_2$, both hydrolysing a phosphatidylcholine substrate. 3. Sphingomyelin [20% molar (20mol of sphingomyelin/80mol of phosphatidylethanolamine)] activates phosphatidylethanolamine hydrolysis by intestinal mucosal phospholipase A_2, and then at higher concentrations (40% molar) substantially inhibits the activity. 4. The results are discussed in relation to possible molecular reorganizations brought about in the hydrated phospholipid substrate complex, and in particular the possible stabilizing role of sphingomyelin in the maintenance of membrane structure, and hence in the modulation of phospholipase activity.

Mammalian cells contain an abundance of intracellular phospholipases, only some of which require Ca^{2+} as an essential cofactor (Van den Bosch, 1980). Their potential activity is such that unrestrained they could very rapidly destroy the membrane integrity. The factors which control such phospholipases are not well understood (Van den Bosch, 1980; Dawson, 1982; Irvine, 1982), although the cytoplasmic level of Ca^{2+} clearly may be important for the Ca^{2+}-dependent enzymes. However, in certain cell systems there are radical differences in the response of intracellular phospholipases to a biological activator (e.g. thrombin on platelets) compared with those produced by the elevation of cell Ca^{2+} concentration brought about with the ionophore A23187 (Lapetina & Cuatrescasas, 1979; Broekman *et al.*, 1980; Rittenhouse-Simmons, 1981). We have made the suggestion that the three-dimensional organization of phospholipid molecules within cell membranes can dramatically alter their susceptibility to intracellular phospholipases (Dawson, 1982; Dawson *et al.*, 1983*a*). In particular, the normal arrangement of phospholipid molecules within a tightly packed bilayer is almost totally resistant to enzyme attack. Any perturbation in the bilayer, which can often be mimicked by diacylglycerol incorporation (Hoffman & Majerus, 1982; Dawson *et al.*, 1983*b*) and which may perhaps be caused *in vivo* by a local accumulation of this acylglycerol within the membrane (Rittenhouse-Simmons, 1979; Nishizuka, 1983), can cause a rapid activation of phospholipase activity.

Here we show that, when three intracellular phospholipases are stimulated into activity by the addition of diacylglycerol, subsequent addition of phospholipids containing a phosphocholine polar head group produces marked inhibition. Sphingophospholipids are particularly effective in this respect, as are the lysophospholipids prepared from the parent phospholipids.

Abbreviation used: CDTA, cyclohexane-1,2-diaminetetra-acetate.

Experimental

Lipids

Egg phosphatidylcholine (containing predominantly stearoyl and oleoyl fatty acyl moieties) and yeast phosphatidylcholine (oleoyl and palmitoleoyl) were prepared by methods previously described (Dawson, 1958; Irvine *et al.*, 1978). Hydrogenation was carried out by dissolving the phosphatidylcholine (1 mg of phosphorus equivalent) in 1.5 ml of ethanol + 2 ml of light petroleum (b.p. 40–60°C). The solution was shaken with 10 mg of Adams catalyst (Pt_2O) under H_2 at 172.5 kPa (25 lbf/in^2) for 2 h. After centrifugation the hydrogenated phosphatidylcholine was recovered and purified by preparative silica-gel t.l.c. (solvent: chloroform/methanol/acetic acid/water, 65:50:1:4, by vol.). Choline plasmalogen was prepared from the lipid fraction isolated from sheep heart. The lipids were treated for 15 min at 53°C with the methylamine reagent described by Clarke & Dawson (1981) to selectively destroy diacylated phospholipid. The plasmalogen was then isolated and purified by using an alumina column followed by preparative silica-gel t.l.c. [chloroform/methanol/conc. (sp.gr. 0.88) NH_3, 100:45:14, by vol.). It was 96% pure. The lysocholine plasmalogen was prepared by extending the alkaline hydrolysis to 60 min, followed by a similar isolation. Lysophosphatidylcholine (egg) was prepared by using phospholipase A_2 (Dawson *et al.*, 1980), and phosphatidic acid by using phospholipase D (Dawson & Hemington, 1967). Oleoylglycerophosphocholine was purchased (Serdary Research Laboratories, London, Ont., Canada) as were sphingomyelins from brains containing mainly stearoyl and oleoyl moieties (Sigma). Sphingosylphosphocholine was prepared by acid hydrolysis of brain sphingomyelin (0.4 ml of conc. HCl + 1.6 ml of methanol, 2 h, 100°C) followed by preparative t.l.c. (solvent: chloroform/methanol/diethylamine/water, 110:50:8:5, by vol.). 1-2-*sn*-Diacylglycerol was prepared from egg phosphatidylcholine by using the α-toxin of *Clostridium welchii* (Dawson *et al.*, 1983*b*, 1984).

Egg phosphatidylcholine (0.8 mg of P) was suspended in 15 ml of incubation medium containing 0.2 ml of sodium maleate/maleic acid buffer (final concn. 3.3 mM), pH 6.8, 0.033 M-$CaCl_2$ and 5 mg of Burroughs–Wellcome α-toxin of *Cl. welchii*. After 2 h incubation at 30°C, 75 ml of chloroform/methanol (2.1, v/v) was added and, after shaking, the lower phase was collected. The lower chloroform-rich phase was shaken with chloroform/methanol/0.1 M-CDTA (3:5:45, by vol.), pH 7, and finally twice with the same solvent mixture containing 0.9% NaCl in place of CDTA. The residue recovered on evaporation was passed down a column (8 cm × 2 cm diameter) of silicic acid and the first 75 ml was collected. Diacylglycerol appeared to be the sole neutral lipid product when monitored by t.l.c., and its concentration was determined by weighing and acyl ester determinations.

The preparations were devoid of phospholipase C activity, Diacylglycerols were purified if necessary on a silicic acid column (Mallinckrodt, St. Louis, MO, U.S.A.) 12 cm × 12 cm diameter, activated for 20 h at 110°C). The column was poured in hexane, 10 mg of diacylglycerol was inserted and the column eluted successively with 100 ml portions of mixtures of hexane/diethyl ether (20:1, 9:1, 17:3, 3:1, 7:3, 2:2, v/v). The diacylglycerol usually emerged in the 3:1–7:3 region and was collected for monitoring by t.l.c. (hexane/diethyl ether/acetic acid, 50:50:1, by vol.). The *sn*-1,3-diacylglycerol travels just ahead of the *sn*-1,2-diacylglycerol. If too extensive (50%) acyl migration had occurred during preparation or storage, the sample was rejected.

Phospholipases

The preparation of tissue extracts for the examination of phosphatidylinositol phosphodiesterase and phospholipase A_2 have been described (Dawson *et al.*, 1980, 1983*b*). Human blood platelets were prepared by filtration through Sepharose (Rittenhouse-Simmons & Deykin, 1976). Rat liver large-granule soluble proteins were isolated as a source of lysosomal phospholipases (Irvine *et al.*, 1978).

The method of [^{32}P] phospholipid preparation and of assaying phospholipase activities has been briefly described previously (Dawson *et al.*, 1983*b*). The ^{32}P-labelled yeast phosphatidylcholine or phosphatidylinositol was mixed in chloroform solution with the lipid additions (diacylglcerol, phosphatidylcholine and sphingomyelin). The mixture was evaporated to dryness *in vacuo* and the lipids then suspended in the aqueous incubation medium by vigorous shaking (Whirlimix). In some instances the suspensions were sonicated until clear in a bath (Decon; Ultrasonics Ltd., Hove, Sussex, U.K.) or probe-type (Mullard, 1.5 A) sonicator, but since this gave essentially the same results and led to some decomposition of the lipid mixture it was not carried out as a routine.

Phosphatidylinositol phosphodiesterase was assayed with an incubation medium (1 ml) containing [^{32}P]phosphatidylinositol (0.33 μmol), 0.02 M-KOH/maleic acid buffer, pH 6.8 (brain enzyme) or 7.4 (platelet enzyme), 0.08 M-KCl (with the brain enzyme only) and 1 mM-$CaCl_2$; 0.05 ml of the rat brain or human platelet extracts was added. Incubation was for 15 min at 37°C, then the incubation mixture was inserted in a boiling-water

bath for 2min and then extracted successively with 1ml of butan-1-ol and 2 × 1ml of diethyl ether. A 0.5ml sample was retained for radioactivity counting; numerous examinations by high-voltage paper ionophoresis (at pH3.6) indicated that the sole ^{32}P-labelled products were inositol monophosphate and inositol cyclic monophosphate, indicative of phosphodiesterase activity only.

The rat intestinal mucosa phospholipase A_2 was assayed in an incubation medium (0.6ml) containing ^{32}P-labelled yeast phosphatidylcholine (0.33mol), 0.017M-Tris/maleate buffer, pH7.4, 2.7mM-EDTA and 0.06ml of intestinal-mucosa extract. Incubation was 30min at 33°C; the latter temperature avoided denaturation of the enzyme, which occurred at 37°C, and ensured virtually linear kinetics. Under these incubation conditions the lysophosphatidylcholine produced was extensively deacylated by a phospholipase B, producing glycerophosphocholine. However, if this latter activity was suppressed with sodium deoxycholate (1mg/mg), then use of phosphatidylcholine substrate specifically labelled in its acyl groups showed that only one acyl glycerophosphocholine was produced (phospholipase A_2 activity). After incubation the tubes were immersed in a boiling-water bath for 2min and then, after cooling, extracted with 1ml of butanol followed by 2 × 1ml of diethyl ether. The aqueous extract was counted for radioactivity, and paper ionophoresis at pH3.6 and chromatography indicated that the ^{32}P-labelled product was exclusively glycerophosphocholine. The solvent extracts were mixed with 4vol. of ethanol, and evaporated to dryness *in vacuo*. The phospholipids were applied to a silica-gel t.l.c. plate, 0.16mol of lysophatidylcholine was applied as a carrier and the chromatogram developed in chloroform/methanol/diethylamine/water (110:50:6:5, by vol.). The lysophosphatidylcholine and phosphatidylcholine spots were located by spraying with a P-detecting reagent (Vaskovsky & Kostetsky, 1968), scraped into scintillation fluid, and the ^{32}P radioactivity counted. The lysophosphatidylcholine recovered invariably contained negligible ^{32}P compared with the glycerophosphocholine produced.

Lysosomal phospholipase A was assayed in a similar manner, except the incubation medium contained 0.033M-sodium acetate/acetic acid buffer, pH4.5, and the incubation was for 30min at 37°C. In this instance, analysis of the incubation products showed that lysophosphatidylcholine was predominant, whereas further deacylation was minimal.

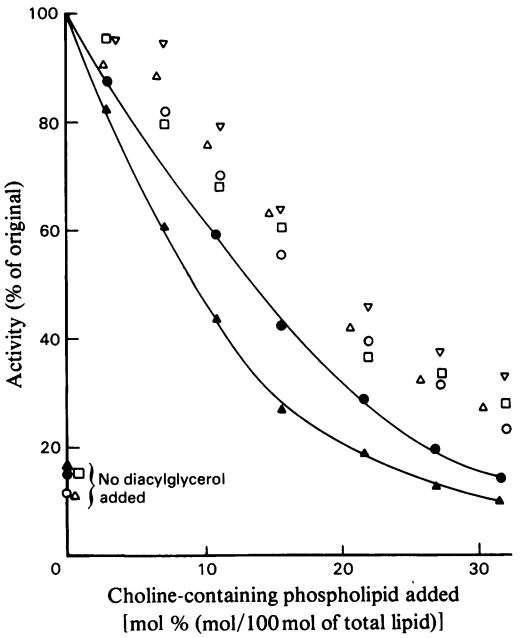

Fig. 1. *Inhibition of diacylglycerol-stimulated rat brain phosphatidylinositol phosphodiesterase by various choline-containing phospholipids*

To the [^{32}P] substrate was added 21%-molar diacylglycerol (egg) to achieve maximum activation (a selection of control values are indicated on the Figure). Sequential additions of various choline-containing phospholipids were added to this mix. The water-soluble radioactivity liberated was determined; this was found in inositol monophosphate and inositol cyclic phosphate, and deacylating activity was negligible. Key to symbols: △, ox heart choline plasmalogen; ○, hen's-egg phosphatidylcholine; □, hydrogenated hen's-egg phosphatidylcholine; ▽, yeast phosphatidylcholine; ●, oleoyl sphingomyelin; ▲, stearoyl sphingomyelin (brain).

Results

Phosphatidylinositol phosphodiesterase

Under the conditions of assay, addition of diacylglycerol (21.5% molar) prepared from egg phosphatidylcholine to the substrate caused a 5–6-fold stimulation of rat brain phosphatidylinositol phosphodiesterase (Figs. 1 and 2). This is similar in magnitude to that which we have observed previously with human platelet enzyme (Dawson *et al.*, 1983*b*). The addition of choline phospholipids containing two long hydrophobic chains (Fig. 1) or choline lysophospholipids containing one long hydrophobic chain (Fig. 2) produced a total inhibition of the activation which had been produced by diacylglycerol. The two sphingomyelins tested, i.e. brain (largely stearoyl) and oleoyl sphingomyelin, were more effective at inhibiting the system than were the choline phosphoacylglycerol group, i.e. egg and yeast phosphatidylcholine, hydrogenated egg phosphatidylcholine

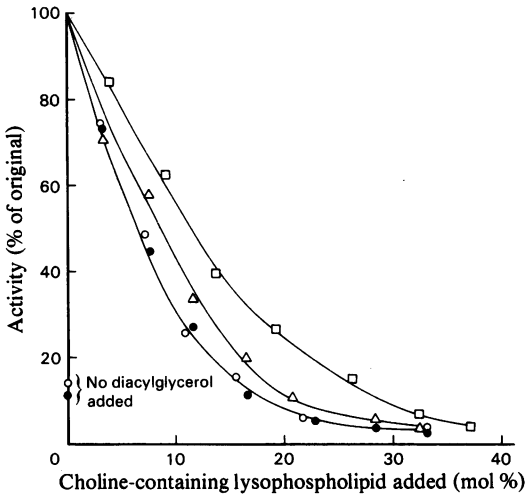

Fig. 2. *Inhibition of diacylglycerol-stimulated rat brain phosphatidylinositol phosphodiesterase by various choline-containing lysophospholipids*

Conditions were as described in the legend to Fig. 1. Key to symbols: ○, hen's-egg lysophosphatidylcholine; □, oleoylglycerophosphocholine; △, lysocholine plasmalogen (alkenylglycerophosphocholine); ●, sphingosylphosphocholine.

and heart choline plasmalogen. On a molar basis the lysophospholipids were appreciably more effective at producing inhibition than the original phospholipids, especially in the phosphoacylglycerol group. Thus 50% inhibition was produced by 18%-molar egg phosphatidylcholine, but only 7% of the corresponding lysophosphatidylcholine was required; again, 18.5% of choline plasmalogen was needed for 50% inhibition, whereas its lyso derivative produced the same inhibition at 8.5% molar. In general, the more unsaturated the hydrophobic chains were in a given phospholipid, the less effective they were at producing inhibition, e.g. stearoyl sphingomyelin was a better inhibitor than oleoyl sphingomyelin, and stearoyl lysophosphatidylcholine more effective than the oleoyl compound. However, this effect was far less marked than the difference between single-hydrophobic-chain and double-hydrophobic-chain phospholipids.

The phosphatidylinositol phosphodiesterase activity assayed displayed linear kinetics with time and against enzyme concentration at the values used; in inhibited systems there was no evidence of the development of a lag phase or rapid fall off of activity, and the lower rate of hydrolysis remained linear up to the full incubation time.

Human platelet phosphatidylinositol phosphodiesterase stimulated with diacylglycerol showed a similar response to the choline-containing phospholipids (Fig. 3), with sphingomyelin producing a powerful inhibition: 50% at 6.5%-molar sphingo myelin added, and 97% at 20% molar. Phosphatidylcholine was less effective, phosphatidic acid had little effect and phosphatidylethanolamine produced a small stimulation of the hydrolysis (Fig. 3).

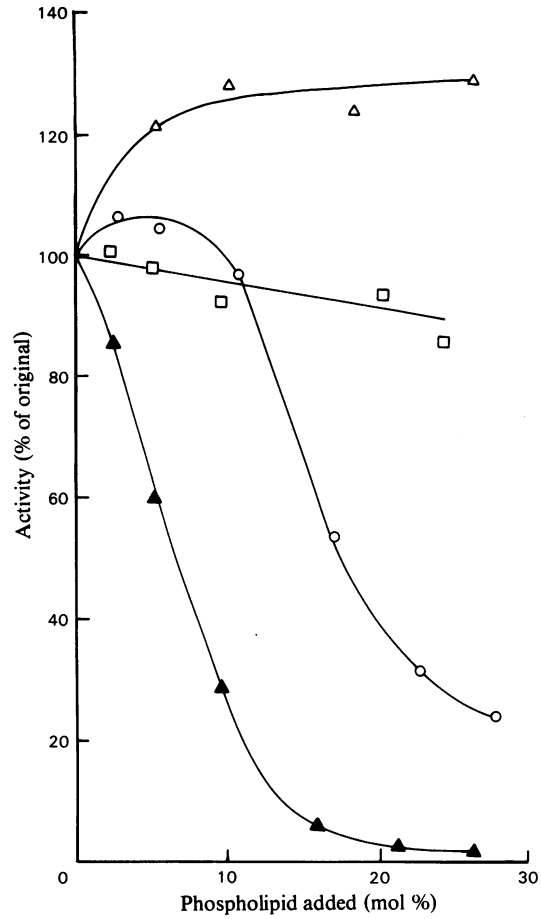

Fig. 3. *Effect of various phospholipids on the diacylglycerol-stimulated phosphatidylinositol phosphodiesterase of human blood platelets*

To the [^{32}P]phosphatidylinositol substrate was added 19.5%-molar diacylglycerol to achieve maximum activation. Sequential additions of various other phospholipids were added to the mix. Water-soluble ^{32}P liberated was measured; no deacylating activity was apparent. Key to symbols: △, yeast phosphatidylethanolamine; □, phosphatidic acid (egg); ○, phosphatidylcholine (egg); ▲, sphingomyelin (brain).

Experiments were carried out to see whether diacylglycerol would stimulate platelet phosphatidylinositol phosphodiesterase when the substrate was incorporated into a lipid mix that corresponded approximately to the composition

obtained by analysis of the inner lamellae of the bilayer in the hepatocyte plasma membrane (Higgins & Evans, 1978) and, if so, the effect of increasing the sphingomyelin level. Under the conditions used, diacylglycerol at 13% molar stimulated the activity 6-fold (Fig. 4). If, at this level of diacylglycerol, the sphingomyelin content of the mix was increased from 9.9% to 15.7%, there was a progressive fall in activity (Fig. 4, inset); 14% sphingomyelin instead of 9.9% in the mix produced 50% inhibition.

Phospholipases A

Phospholipase A_2 of rat intestinal mucosa shows little ability to hydrolyse phosphatidylcholine alone, but diacylglycerol addition produces activation of the enzyme (Dawson *et al.*, 1983*b*). Added sphingomyelin inhibits the diacylglycerol-stimulabed enzyme strongly (Fig. 5); e.g. 6% of this phospholipid causes 50% inhibition.

No lag phase or rapid fall off of activity was apparent, and the lower rate showed linear kinetics up to the incubation time employed.

A preparation of liver lysosomal phospholipase A (probably a mixture of A_1+A_2) attacking phosphatidylcholine at pH 4.5 was also found to be activated by diacylglycerol; 18% of the latter lipid increased lysophosphatidylcholine release 6-fold. Sphingomyelin caused a marked inhibition of this diacylglycerol-stimulated activity (Fig. 6). This lysosomal phospholipase A would readily attack a phosphatidylethanolamine substrate on its own (cf. Robinson & Waite, 1983); indeed, diacylglycerol caused an inhibition of the attack. Sphingomyelin addition, however, produced a marked activation of the phosphatidylethanolamine hydrolysis, and it was only at much higher concentrations that inhibition of the system was produced (Fig. 6). The likely reason for this is that sphingomyelin is initially (at low concentrations) promoting the formation of a lipid structure intermediate between a true hexagonal phase and a bilayer (Das & Rand, 1984; Dawson *et al.*, 1984), which is favoured by other phospholipases (Dawson *et al.*, 1984), but at higher concentrations sphingomyelin causes a bilayer (a non-favoured substrate structure) to be formed.

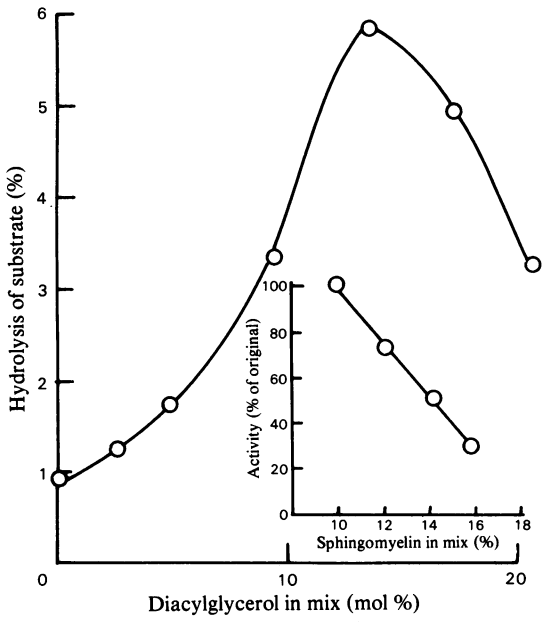

Fig. 4. *Activation by diacylglycerol and inhibition by sphingomyelin of phosphatidylinositol phosphodiesterase when the substrate is incorporated in a mix corresponding to plasma-membrane phospholipids*

The [^{32}P]phosphatidylinositol was incorporated into a mixture of phospholipids and cholesterol whose composition corresponded to the inner lamellae of the hepatocyte plasma-membrane bilayer (Higgins & Evans, 1978; Dawson *et al.*, 1980). Diacylglycerol was added sequentially and the susceptibility to human platelet phosphatidylinositol phosphodiesterase determined by measuring the water-soluble ^{32}P liberated. In further experiments the system was stimulated to the maximum with diacylglycerol (13% molar) and the sphingomyelin content of the mix increased from the control 9.7% molar (inset).

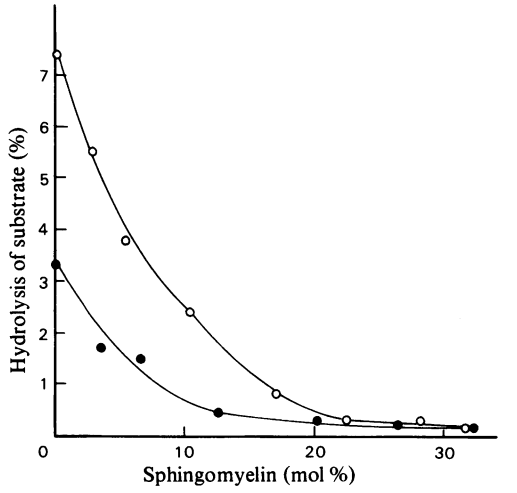

Fig. 5. *Inhibition by sphingomyelin of diacylglycerol-stimulated intestinal phospholipase A_2 attacking phosphatidylcholine*

[^{32}P]Phosphatidylcholine was mixed with either 17.3% molar diacylglycerol (●) or 26.6% diacylglycerol (○), the latter level giving maximum activation. Sphingomyelin (brain) was sequentially added to these mixes and the extent of the deacylation determined (see the Experimental section).

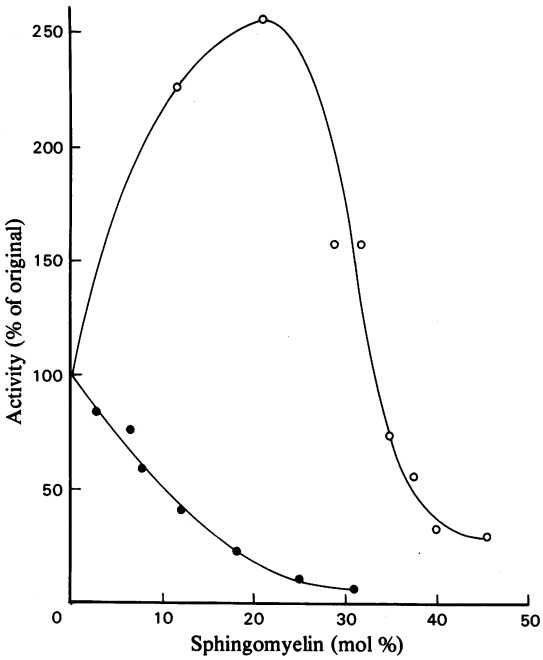

Fig. 6. *Effect of sphingomyelin on the deacylating phospholipases ($A_1 + A_2$) of a rat liver lysosome fraction*
The substrates were (●) [^{32}P]phosphatidylcholine plus 17.2%-molar diacylglycerol (maximum stimulation) or (○) [^{32}P]phosphatidylethanolamine. Sphingomyelin (brain) was added sequentially to these substrates and the susceptibility to lysosomal phospholipases ($A_1 + A_2$) determined by measuring the [^{32}P]lysophospholipid released plus any water-soluble ^{32}P-labelled components.

Discussion

It is now becoming evident that diacylglycerol has a unique stimulating effect on many phospholipases when these are attacking a phosphatidylcholine or a phosphatidylinositol substrate (Hoffman & Majerus, 1982; Dawson *et al.*, 1983*b*, 1984). Its action is not mimicked by triacylglycerol, and activators such as fatty acids, monoacylglycerol and deoxycholate are only effective at much higher concentrations (Dawson *et al.*, 1984; R. M. C. Dawson, N. Hemington & R. F. Irvine, unpublished work). The present report that a mixed phospholipase $A_1 + A_2$ from rat liver lysosomes is also markedly stimulated by diacylglycerol extends the list of intracellular phospholipases which show this phenomenon, and emphasizes the probability that its action is most likely to be in its ability to change the organization or orientation of the molecules comprising the hydrated phospholipid substrate. There is no evidence that the action of diacylglycerol merely causes a dispersion of the phosphatidylcholine into finer particles; indeed, the activation is still observed with substrate particles that have been dispersed by sonication to produce a water-clear colloidal solution. Furthermore, examination by ^{31}P n.m.r. has recently demonstrated that diacylglycerol addition causes an isotropic component to develop in the typical phosphatidylcholine bilayer spectrum which approximately correlates with the advent of enzymic hydrolysis (Dawson *et al.*, 1984).

In previous investigations it has been noted that the non-stimulated activity of phosphatidylinositol phosphodiesterase can be inhibited by phosphatidylcholine and sphingomyelin (Irvine *et al.*, 1979; Dawson *et al.*, 1980; Hoffman & Majerus, 1982). The present results clearly indicate that all phospholipids with a polar head group containing a phosphocholine residue are antagonistic to the activating effect of diacylglycerol on this enzyme. A probable explanation is that they reverse the diacylglycerol-produced perturbation in the bilayer structure revealed by ^{31}P n.m.r., so that the phospholipid substrate reverts to the classical bilayer form (smectic mesophase) resistant to intracellular phospholipases. If this is so, it is perhaps surprising that with the phosphatidylinositol phosphodiesterase the lysophospholipids such as lysophosphatidylcholine, alkenylglycerophosphocholine and sphingosylphosphocholine are twice as effective inhibitors on a molecular basis than are the parent phospholipids. These lysophospholipids are often regarded as wedge-shaped molecules which, at high enough concentrations, can perturb the bilayer structure, often forming complex micelles. It is probable that at lower concentrations they can have a specific stabilizing effect on the perturbed structure, perhaps by virtue of their phosphocholine-containing head group. Thus, since they contain only one hydrophobic chain and occupy less space in a monolayer (or single lamellae of a bilayer) at a given surface pressure, then their concentration in a unit area of the mixed-substrate surface would be greater than that of the parent choline-containing phospholipids.

It is known that the activity of many phospholipases can be influenced by the zeta potential existing near to the substrate's surface (Dawson *et al.*, 1976). Since phospholipids containing phosphocholine head groups are zwitterions, they would not neutralize the surface charge on the phosphatidylinositol substrate particles, although possibly some small dilution of the charge density could occur. However, in other studies (R. M. C. Dawson, N. Hemington & R. F. Irvine, unpublished work) we have observed a lack of inhibition by other neutral spacer molecules or the reversal of the inhibition by the addition of anionic amphipathic molecules, suggesting that this mechanism

for the inhibition is unlikely. Diacylglycerol itself activates many phosphatidylcholine-attacking enzymes without inducing any electrokinetic changes in the substrate (Dawson *et al.*, 1984).

The indications are that sphingomyelin is a more effective inhibitor of phosphatidylinositol phosphodiesterase than is phosphatidylcholine or choline plasmalogen. More importantly, sphingomyelin causes marked inhibition of the diacylglycerol-stimulated phosphatidylcholine breakdown brought about by the deacylating phospholipase of intestinal mucosa or rat liver lysosomes. A possible explanation must be that sphingomyelin is a more effective stabilizer of the bilayer structure than is phosphatidylcholine against the perturbing effect of diacylglycerol and the attack of phospholipases. Alternatively sphingomyelin might have a more direct effect on the active centre of the enzyme, combining with this and blocking its reaction with the phosphatidylcholine substrate, although deacylation of sphingomyelin is not observed. However, our observations that sphingomyelin inhibits two quite different phospholipase enzymes (in the present study) and similar evidence we have regarding the activity of other phospholipases (R. M. C. Dawson, N. Hemington & R. F. Irvine, unpublished work), would suggest a different explanation given the known high specificity of these enzymes for the hydrophobic region of their substrates. Whether or not sphingomyelin has a specific function in cell membranes is unknown, although it is usually a significant component of the phospholipid bilayer and occurs in especially high concentrations in the plasma membrane. A surfeit of sphingomyelin, e.g. in the infantile form of Niemann–Pick disease (Gatt *et al.*, 1978), causes severe mental retardation and ultimately a vegetative state and death. A further observation relevant to the unique properties of sphingomyelin is that Gatt & Bierman (1980) have shown that sphingomyelin, as distinct from phosphatidylcholine, can cause a suppression of the binding of low-density lipoproteins to surface receptors on culturered skin fibroblasts.

Recently it has been shown that the biosynthesis and concentration of sphingomyelin can be enhanced in cells by glucocorticoids, for example leucocytes (Nelson *et al.*, 1982), fat-cell ghosts (Murray *et al.*, 1979) and HeLa cells (Johnston *et al.*, 1980). It is generally accepted that these anti-inflammatory steroids operate by inhibiting deacylating phospholipases, thus suppressing arachidonic acid release and the subsequent synthesis of prostaglandins (Blackwell & Flower, 1983). Evidence is available that they do this by promoting the release of various anti-phospholipase proteins such as macrocortin (Blackwell *et al.*, 1980) or lipomodulin (Hirata, 1981). However, it could be that the enhanced content of sphingomyelin also plays some role by toning down the increased action of various cellular phospholipases brought about by perturbations in the bilayer structure. This perturbation could be through diacylglycerol accumulation in response to receptor activation (Rittenhouse-Simmons, 1979; Nishizuka, 1983, 1984) or through other factors, as yet unknown, which bring about the release of arachidonic acid and prostaglandin synthesis in response to stimulation (Irvine, 1982). Certainly our present results show that if, in an artificial plasma membrane (Fig. 4), the sphingomyelin content is increased by a similar magnitude to that induced by glucocorticoids in intact cells (see above), a significant fall in phospholipase activity is the result.

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