THE EXOGENOUS LIPID REQUIREMENT FOR HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS STIMULATED BY CONCANAVALIN A

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1. Introduction

Mast cells contain storage granules which fuse with the plasma membrane and release their contents from the cell when stimulated in vivo by an antigen to which the animal has been sensitised [1,2]. This process is responsible for the anaphylactic response, and the main component of the storage granules is histamine. The mechanism of stimulation involves the crosslinking of the IgE molecules bound to F_c receptors on the cell surface [3]. Dimerisation of the receptors appears to generate a sufficient signal, since stimulation by dimeric IgE is as effective as polymeric forms of the antibody [4]. The stimulation of the cells in vitro occurs with a wide range of agents which interact with the plasma membrane, including the plant lectin concanavalin A (con A) [5,6]. Con A appears to act by the same mechanism as antigen in that it crosslinks IgE molecules bound to the cell surface by multivalent interaction with carbohydrate residues on the IgE molecules [7].

A peculiarity of the rat peritoneal mast cell system in vitro is that stimulation by antigen [8], anti-IgE antibody [9], con A [6] and anti F_c receptor antibody [10] is strongly and specifically potentiated by the lipid phosphatidyl serine (PS). Here we report further examination of the lipid involvement in the stimulation of mast cells by con A. We show that the lyso-PS analogue is at least 10^3-fold more potent than PS; that it probably acts by binding directly to the cells, and that $<3 \times 10^6$ molecules/cell of bound lyso-PS are required for maximal co-stimulation with con A. Although the entry of Ca^{2+} into the cells has been strongly implicated in the mechanism of stimulation [11], lyso-PS appears to have little or no action as a Ca^{2+} ionophore by itself or in combination with the Ca^{2+} ionophore A23187 which stimulates mast cells in the presence of external Ca^{2+} [12].

2. Materials and methods

2.1. *Cell preparation and assays*

Rat peritoneal mast cells were prepared by the method in [6]. The cells were subsequently washed thoroughly in medium without 0.1% bovine serum albumin (NaCl 150 mM; KCl 3.7 mM; Na_2HPO_4 3.0 mM; KH_2PO_4 3.5 mM; $CaCl_2$ 0.9 mM; glucose 5.6 mM; gelatin 0.1% w/v; heparin 10 U/ml; pH 6.8). Female Wistar rats (200–300 g) were routinely used, although parallel experiments with Sprague-Dawley and Hooded Lister rats using both sexes of each strain gave similar responses. The total cell yields varied by up to 5-fold with sex and strain.

The cells were exposed only to surfaces of polyethylene, polystyrene, or glass siliconised with 'Repelcote' (BDH). The cells (10^5/ml) were incubated with reagents for 15 min at 25°C. Con A stock solutions in medium were centrifuged to clarity before addition to 100 $\mu g/ml^{-1}$ final conc. To avoid the problems associated with the reproducible addition of the insoluble calcium ionophore A23187, a stock solution of the magnesium complex of the ionophore (1 mg/ml) in liposomes of dioleoyl phosphatidyl choline (10 mg/ml) was prepared by ultrasonication in 10 mM sodium acetate buffer (pH 7.0) containing 2 mM magnesium sulphate. In this form the ionophore solution is stable indefinitely when frozen. The

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appearance of the cells was monitored throughout by microscopy, and cell lysis was determined by assaying lactate dehydrogenase activity in the medium by NADH oxidation. Histamine release was assayed as in [6], using an Aminco Bowman spectrofluorimeter. Control experiments were performed in parallel to correct for the effects of cholesterol, phospholipids or A23187 on the assay. All chemicals were Analar grade (BDH); bovine serum albumin (grade III) was obtained from Sigma, and gelatin from Harrington Biochem. The calcium ionophore A23187 was generously provided by the Lilly Research Centre Ltd.

2.2. *Lipid preparations and analyses*

Dioleoyl and dipalmitoyl phosphatidyl choline were prepared by the method in [13]. Phosphatidyl cholines were converted to the corresponding phosphatidyl serines by treatment in ether with phospholipase D in the presence of a standard aqueous solution of serine in a sealed vessle at 40–45°C [14]. The phosphatidyl serines were isolated by absorption onto Whatman DE 32 in chloroform–methanol (2:1, v/v) and specifically eluted with 50% acetic acid in the same solvent. Lyso-lipids were prepared by treating the lipids with cobra venom (*Naja naja*). The liberated fatty acid was removed by ether extraction and the venom protein by chloroform precipitation after drying. [^{3}H]Lyso-PS (110 mCi/mmol) was prepared by exposure of bovine PS (20 mg) in dioxan solution to tritium gas (1 Ci) over platinum black catalyst (2 mg) for 4 days with careful exclusion of oxygen, followed by conversion to [^{3}H]lyso-PS as described.

The lipid fatty acid composition of bovine PS was: 18:0, 40.1%; 18:1, 40.3%; 20:1, 14.0% and lyso-PS from bovine PS was 18:0, 83.7%; 18:1, 8.6%; 20:1, 3.6%.

3. Results and discussion

3.1. *Comparison of PS and lyso-PS as costimulants with con A*

Mast cells prepared as described are not stimulated by con A alone to release histamine (<5% in 15 min at 25°C; see fig.1). However, as reported [6], the addition of PS with con A causes a substantial increase in histamine release (50% in 15 min at 10^{-4} M). It is not clear from this observation whether the requirement for PS represents a molecular binding of PS

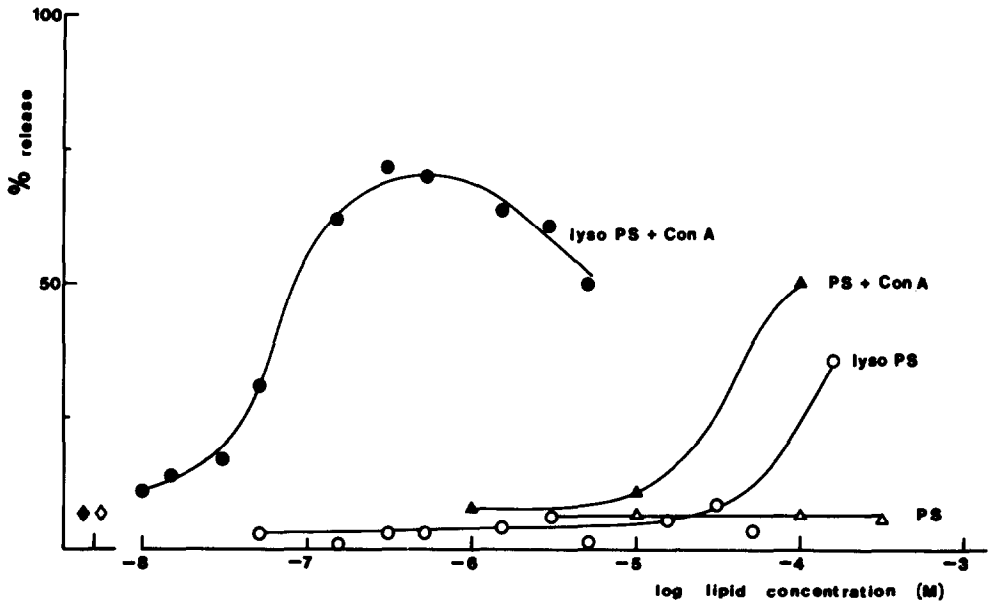

Fig.1. The effect of PS and lyso-PS on histamine release from mast cells (15 min at 25°C) with (▲,●) or without (△,○) con A (100 μg/ml). All release was non-lytic except at 10^{-4} M lyso-PS in the absence of con A. Zero lipid additions with or without con A (◆,◇).

molecules to the cells, or whether vesicles of PS bilayer interact with the cells to produce a surface action which potentiates con A stimulation. Thus the total amounts of PS added are at least 10^6-fold higher than any reasonable estimate of the maximal binding capacity of the plasma membrane for dispersed PS molecules. In addition, the presence of a large excess of PS interferes substantially with measurements of Ca^{2+} uptake into the cells. To resolve these problems we examined the lyso-PS analogue, which has a much higher critical micellar concentration than PS, for the ability to act as a costimulant with con A, and found that it was active at a very much lower total concentration than PS (50% histamine release in 15 min at 10^{-7} M; see fig.1). This concentration of lyso-PS is well below the c.m.c. ($\simeq 10^{-5}$ M) so that the lysolipid is dispersed in solution and presumably equilibrates very rapidly with the plasma membrane of the cells. Similar results with lyso-PS have been reported very recently [16].

Lyso-PS in the absence of con A causes histamine release only at much higher concentrations (25% in 15 min at 10^{-4} M), and this release is entirely due to lysis of the cells, assayed by release of lactate dehydrogenase. Lyso-PS is not therefore able to stimulate the cells by acting independently of con A as a calcium ionophore like A23187.

3.2. *Binding of lyso-PS to the cells*

The action of lyso-PS at 10^{-7} M as a costimulant with con A rules out the possibility that stimulation requires the presence of vesicular bilayers of the lipid, and suggests that direct binding of lyso-PS molecules to the cells is required. Addition of bovine serum albumin (0.1 mg/ml) to the medium shifts the dose response curve for lyso-PS + con A in fig.1 to 10-fold higher lipid concentrations, presumably by lowering the concentration of free lipid in solution.

Attempts were made to assay the binding of [^{3}H]lyso-PS to the cells. No depletion of ^{3}H counts from the supernatant after centrifugation of the cell suspension could be detected on addition of lyso-PS from 5×10^{-8} M to 5×10^{-6} M at a cell density of 10^6 cells/ml in the presence or absence of con A (fig.2). At the lowest concentration of lyso-PS for maximal stimulation ($\simeq 10^{-7}$ M) <5% of the lyso-PS is associated with the cells, which corresponds to $< 3 \times 10^6$ molecules/cell. However a further possibility

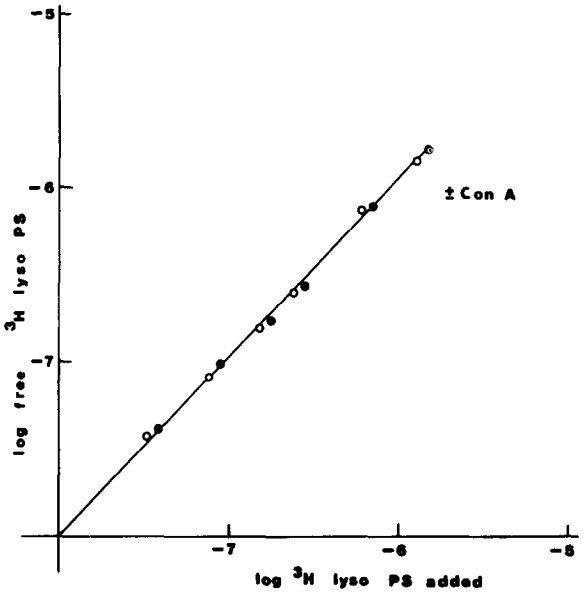

Fig.2. [^{3}H]Lyso-PS in the supernatant after centrifugation of the cells (10^6/ml) as a function of the total lyso-PS added with or without con A (●,○).

is that the lyso-PS coats the surface of the polyethylene tubes to provide a surface interaction with the cells to trigger the con A response. A sequence of 6 transfers of the optimal (10^{-7} M) concentration of lyso-PS from tube to tube depleted the total [^{3}H]-lyso-PS counts by <5%, indicating that the average surface density of lyso-PS molecules is $< 2.5 \times 10^5 / 2.5 \times 10^{-10}$ m^2 (the approximate area covered by a cell). It is clear that accurate estimates of binding of lyso-PS directly to the cells and to the surfaces of the tubes are required.

The radioactivity was quantitatively recovered after con A stimulation of cells in the presence of 10^{-8} M or 10^{-7} M [^{3}H]lyso-PS by chloroform–methanol extraction of the freeze dried incubations. Silica gel chromatography [14] of the lipid extract failed to detect any metabolism of the [^{3}H]lyso-PS.

3.3. *Specificity of lyso-lipid costimulation*

No difference was observed in the dose–response curves of lyso-PS prepared from beef brain PS, dioleoyl or dipalmitoyl PS. However the presence of a fatty acid chain required: no costimulation was obtained with glycerophosphoryl serine (GPS) over

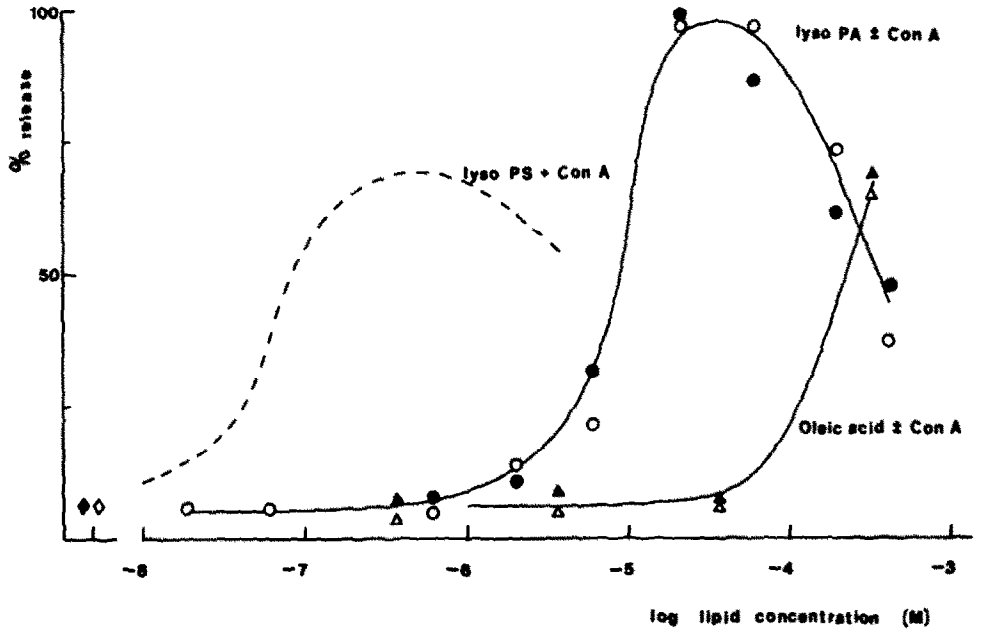

Fig.3. The effect of oleic acid and lyso-oleoyl phosphatidic acid on histamine release by cell lysis with (▲,●) or without (△,○) con A (100 μg/ml). The dashed curve is for the non-lytic release of histamine by lyso-PS + con A taken from fig.1 for comparison. Zero lipid additions with or without con A (◆,◇).

10^{-6}–10^{-3} M, presumably because it is unable to interact with the cell membranes, and GPS did not act as a competitive inhibitor of lyso-PS costimulation with con A over the same concentration range.

The dose–response curves for lyso-phosphatidic acid and oleic acid with and without con A are shown in fig.3. No synergistic effect of con A is observed with either agent indicating that the mechanism of release is by cell lysis, which was confirmed by measurement of lactate dehydrogenase release. A range of other lyso-phospholipids were tested, including lyso-phosphatidyl inositol, lyso-phosphatidyl ethanolamine, lyso-phosphatidyl glycerol, and lyso-phosphatidyl choline and found to be inactive as costimulators with con A. Taken together, these observations support the hypothesis that a specific molecular interaction between lyso-PS or PS and the plasma membrane is required for the stimulation of histamine release by con A.

3.4. *Does lyso-PS act as a calcium ionophore?*

The simplest hypothesis for the mechanism of mast cell stimulation is that the entry of extracellular calcium into the cytoplasm is the essential triggering event. This view is supported by the energy-dependent, non-lytic release of histamine stimulated by the calcium ionophore A23187 in the presence of extracellular calcium [12]. Stimulation by A23187 does not require the presence of exogenous PS. One possible mechanism for the costimulating action of lyso-PS is that it might carry Ca^{2+} into the cells by forming a neutral lyso-PS–Ca^{2+} (2:1) complex, analogous to the well-defined complex of A23187 and Ca^{2+} [15]. However, it has already been shown that lyso-PS has no ionophore-like activity by itself, in that it only releases histamine by cell lysis in the absence of con A (fig.1). In addition, it can be seen from fig.4 that 10^{-5} M lyso-PS had no significant effect on release mediated by A23187, confirming that any activity of lyso-PS as an independent ionophore for calcium is very weak. It should be emphasised, however, that this does not exclude the possibility that a complex of lyso-PS with crosslinked IgE receptors functions as a calcium transport system.

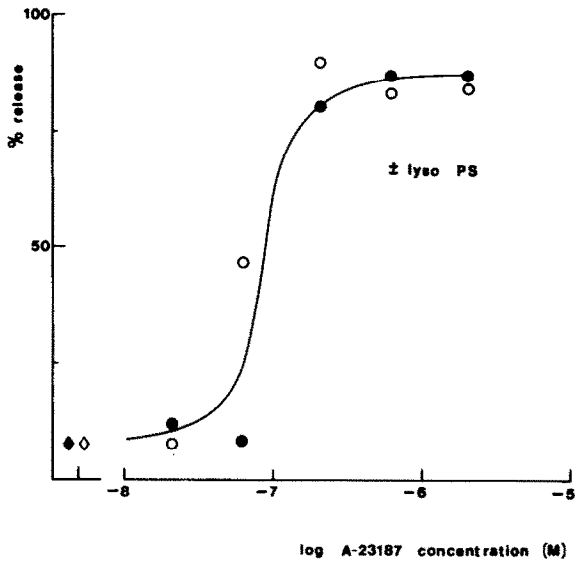

Fig.4. The effect of the calcium ionophore A23187 and 1 mM calcium on non-lytic histamine release with (•), or without (○) lyso-PS (10^{-5} M). Zero ionophore addition with or without 10^{-5} M lyso-PS (♦,◇).

4. Conclusions

1. Lyso-PS is ~10^3-fold more potent than PS as a costimulator with con A of histamine release from mast cells. The action of lyso-PS probably involves direct binding of the lipid molecules to the cell; interaction with lipid bilayer or micellar structures does not appear to be required. Less than 3×10^6 molecules of lyso-PS bound per cell are required for maximal costimulation with con A. There is no detectable metabolism of the lipid during the assay period for histamine release.
2. Of the lipids examined, only lyso-PS acted as a non-lytic, costimulant with con A: in the absence of con A lyso-PS at higher concentrations caused cell lysis. Other lyso-lipids and oleic acid with or without con A had no effect below lytic concentrations. An acyl chain was necessary for lyso-PS to have activity, but activity was not affected by chain length or saturation ($14 < C_n < 18$).
3. Lyso-PS had no detectable activity as an independent calcium ionophore when used alone or in combination with A23187 as a stimulating agent.

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References

[1] Goth, A. and Johnson, A. R. (1975) Life Sci. 16, 1201–1214.
[2] Lawson, D., Raff, M. C., Gomperts, B., Fewtrell, C. and Gilula, N. B. (1977) J. Cell Biol. 72, 242–259.
[3] Ishizaka, T. and Ishizaka, K. (1978) J. Allergy Clin. Immunol. 61, 320–330.
[4] Segal, D. M., Taurog, J. D. and Metzger, H. (1977) Proc. Natl. Acad. Sci. USA 74, 2993–2997.
[5] Keller, R. (1974) Clin. Exp. Immunol. 13, 139.
[6] Sullivan, T. J., Greene, W. C. and Parker, C. W. (1975) J. Immunol. 115, 278–282.
[7] Siraganian, R. P. (1976) in: Mitogens in Immunobiology (Oppenheim, J. J. and Rosenstreich, D. L. eds) pp. 69–84, Academic Press, New York.
[8] Goth, A., Adams, H. R. and Knoohuizen, M. (1971) Science 173, 1034–1035.
[9] Baxter, J. H. and Adamik, R. (1978) Biochem. Pharmacol. 27, 497–503.
[10] Ishizaka, T. and Ishizaka, K. (1978) J. Immunol. 120, 800–805.
[11] Foreman, J. C. and Mongar, J. L. (1975) in: Calcium Transport in Contraction and Secretion (Carafoli, E. et al. eds) pp. 175–184, Elsevier/North-Holland, Amsterdam, New York.
[12] Foreman, J. C., Mongar, J. L. and Gomperts, B. D. (1973) Nature 245, 249–251.
[13] Robles, E. and van den Berg, D. (1969) Biochim. Biophys. Acta 187, 520–526.
[14] Smith, G. A., Montecucco, C. and Bennett, J. P. (1978) Lipids 13, 92–94.
[15] Deber, C. M. and Pfeiffer, D. R. (1976) Biochemistry 15, 132–141.
[16] Martin, T. W. and Lagunoff, D. (1979) Nature 279, 250–252.