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The effect of phospholipids on anaphylactic histamine release

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Summary

1. Histamine release by antigen from three sensitized rat tissues is potentiated by phosphatidyl serine (PS). The effect is greatest with isolated peritoneal cells. Phosphatidyl inositol, ethanolamine, choline and phosphatidic acid are inactive.

2. PS greatly increases the rate of antigen-induced histamine release and only slightly prolongs the duration of the release process.

3. PS shows a concentration-dependent effect over the range 1–10 μg/ml. At 10 μg/ml it is active over a wide range of antigen concentrations.

4. Calcium ions are necessary for the potentiation by PS of antigen-induced histamine release from rat peritoneal cells.

Introduction

Phospholipids are ubiquitous components of membrane systems. Phosphatidyl serine (PS), usually accounts for less than 10% of the total phospholipid in tissues but it is present in somewhat higher concentrations in plasma membranes and may well have a specific function there. It has been specifically implicated in the ATP-ase activity of the sodium pump by Wheeler & Whittam (1970), and in the release of histamine by macromolecules by Goth and colleagues, who showed that it enhanced the release of histamine from the peritoneal mast cells of certain strains of rats by dextran or ovomucoid (Goth & Adams, 1970) and by antigen (Goth, Adams & Knoohuizen, 1971).

Enhancement of the anaphylactic release of histamine from human leucocytes by uncharacterized factors from normal serum has been demonstrated (Lichtenstein & Osler, 1966 ; Lichtenstein, 1968).

We have been concerned with the action of phospholipids on the anaphylactic release of histamine from rat peritoneal cells. Rat mesentery and lung and guinea-pig lung have also been tested. Preliminary mention of this work has been made (Mongar, 1971 ; Mongar & Svec, 1972).

Methods

Sensitization

Hooded rats 150–250 g of either sex were sensitized according to two schedules: (I) 0·5 ml egg albumen (5 mg/ml in 0·9% NaCl solution) i.m. and 0·5 ml Pertussis Vaccine B.P. (2×10^{10} organisms) i.p. ; (II) 0·5 ml Freund's adjuvant (incomplete) in which was suspended 2·5 mg egg albumen by mixing in a glass

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homogenizer. This was injected intramuscularly and 0·5 ml Pertussis Vaccine B.P. was injected intraperitoneally.

Preparation of peritoneal cells

3 ml of saline, or in some experiments Tyrode solution containing 5 U/ml of heparin, was injected into the peritoneal cavity of a decapitated rat. The abdomen was massaged for 1–2 min and the peritoneal cavity opened by a midline incision. The fluid was then removed from the posterior part of the cavity with a pipette and centrifuged for 5 min at 100 *g* in the cold. In some experiments the mast cells were concentrated by layering the washings over a bovine serum albumen gradient as described by Perera & Mongar (1963). The pellet of cells at the bottom of the centrifuge tube was re-suspended in cold Tyrode solution. The cells from several rats were combined and made up to 4 ml per rat. Aliquots of 0·5 ml were added to centrifuge tubes containing 0·5 ml Tyrode with or without phospholipid. After 5 min pre-incubation at 37° C 0·1 ml antigen solution (usually 1–5 mg/ml of egg albumen) was added and the mixture was incubated at 37° C for varying times. In some experiments the PS was added with the antigen. The reaction was stopped by adding 2 ml ice-cold Tyrode and the tubes were placed in ice, centrifuged in the cold for 10 min at 800 *g* and the supernatant decanted for assay. The pellets were re-suspended in 5 ml Tyrode and boiled for 10 min to release the remaining histamine.

Preparation of tissues

Perfused rat lung was chopped into rods 0·8 mm^2, washed for 10–20 min in a large volume of Tyrode solution, filtered off and 0·15 ml samples prepared (Mongar & Schild, 1956). Rat mesentery was subjected to a similar chopping procedure. The resulting product consisted of small pieces of mesentery which, after washing and sampling, showed a low spontaneous histamine release. The samples of lung or mesentery were suspended in 2 ml Tyrode alone or containing phospholipid. After 5 min incubation 0·2 ml antigen solution was added. After various times the solution was removed from the tissue with a filter pipette, 2 ml of cold Tyrode added and immediately pipetted off. The residual tissue was treated as described above for cells.

Assays for histamine were done on the terminal atropinized (1 ng/ml) guinea-pig ileum. Where necessary phospholipid was added to the histamine standards.

Three phospholipids were obtained from Koch-Light Laboratories Ltd.: phosphatidyl-L-serine (PS) (batch no. 48376) was prepared from bovine brain; phosphatidyl ethanolamine (batch no. 78774) was a synthetic product; phosphatidyl choline (batch no. 54173) was obtained as '43402t L-3-lecithin synthetic pure'. Thin layer chromatograms of PS showed traces of impurities. Amino acid analysis after hydrolysis in 6 N HCl at 110° for 72 h indicated 80% serine, about 10% glycine and traces of glutamate aspartate alanine and leucine. A highly purified phosphatidyl inositol (PI) was kindly supplied by Dr. E. Lea of the University of E. Anglia; 50 μg gave only one spot when run on a thin layer chromatogram and sprayed with phosphomolybdic acid; no amino acids were seen when the thin layer chromatogram was sprayed with ninhydrin. Commercial samples of PI were found to be grossly impure and early results of their biological activity must be attributed to the impurities.

For most experiments suspensions of the phospholipids were prepared by gently homogenizing in Tyrode in a glass cylinder with a close fitting Teflon piston. In one series of experiments the suspensions were also prepared by sonication. The method of preparing the suspension did not affect the activity.

The Tyrode solution used had the following composition: NaCl 137 mM, KCl 2·7 mM, NaH_2PO_4 0·4 mM, $CaCl_2$ 1·8 mM, $MgCl_2$ 1·0 mM, $NaHCO_3$ 12 mM, glucose 5·6 mM. In some experiments the $CaCl_2$ concentration was varied in the range 0·01–10 mM. No adjustment was made to the tonicity of the resulting solutions.

Potentiation (Pot^n) was calculated by the following formula:

$$\frac{\text{(release with antigen + phospholipid) − (release with phospholipid alone)}}{\text{(release with antigen alone) − (spontaneous release)}}$$

Releases are expressed as a percentage of total tissue histamine.

Results

Spontaneous release with phosphatidyl serine (PS) alone

Rat peritoneal cells release histamine spontaneously. In the 3 series of experiments the average release ranged from 3·0 to 5·4% of the total histamine content. In the presence of 100 g/ml PS the rate of release was increased. The increase was small and ranged from 0·4 to 1·7% and appeared to be slightly more for sensitized than normal cells (Table 1). Occasionally quite large releases were obtained but

TABLE 1. *Effect of phosphatidyl serine (PS) (100 μg/ml) on histamine released (% of tissue content) spontaneously and by antigen from various tissues*

Tissue	Spontaneous release			Release with antigen (corrected)		
	Alone	With PS	Pot^n	Alone	With PS	Pot^n
Rat peritoneal cells (8)* Sensitization I	5·4±0·70	6·4±0·88	1·2	4·7±2·2	10·9±3·67	2·3
Rat peritoneal cells (8) Sensitization II	3·3±0·35	5·0±0·72	1·5	12·0±5·8	35·0±5·4	2·9
Rat peritoneal cells (10) Normal	4·3±0·76	4·6±0·73	1·1	—	—	—
Rat mesentery (6)	4·5±1·1	5·7±1·1	1·3	13·3±7·1	21·1±9·9	1·6
Rat lung (5)	2·0±0·33	2·4±0·45	1·2	13·2±2·2	17·0±3·0	1·6 } 1·2
„ „ (9)	2·8±0·41	3·0±0·35	1·1	29·8±3·07	30·5±2·73	1·0 }
Guinea-pig lung (11)	1·5±0·14	2·3±0·67	1·5	38·6±4·39	43·3±5·5	1·1

*No. of experiments

we have not been able to trace the cause of this. The mechanism of the spontaneous release of histamine from peritoneal cells will form the subject of a separate investigation.

Intact tissues (rat mesentery and lung and guinea-pig lung) chopped into small fragments also show a spontaneous histamine release which was similarly increased by PS.

Potentiation of anaphylactic histamine release by phosphatidyl serine

(a) *Rat peritoneal cells.* In 8 experiments animals sensitized 13–15 days previously by schedule I were only weakly sensitized: the average histamine release

with antigen (500 μg/ml) was 10·1%, about twice the spontaneous release (5·4%). In the presence of PS (100 μg/ml) the antigen-induced release was increased to 17·3% with a blank of 6·4%. The resulting potentiation was 2·3 fold (Table 1).

In 8 further experiments with rats sensitized according to schedule II greater anaphylactic releases were obtained, a net 12% (compared with 5% for schedule I) with a similar degree of potentiation by PS (average 2·9 fold, Table 1).

(b) *Rat mesentery.* In 6 experiments the chopped mesentery of sensitized animals (schedule II) released on average 17·8% histamine with antigen compared with 4·5% without. The corresponding values with PS present were 26·8% and 5·7%, giving a potentiation of 1·6 fold.

(c) *Rat lung.* In a series of 5 experiments with weakly sensitized rats, histamine release with antigen was 13·2% above the spontaneous value and was increased to 17·0% in the presence of 100 μg/ml PS. This result was not confirmed in a second series of 9 experiments with more highly sensitized animals (Table 1). Lower concentrations of PS (6 and 25 μg/ml) were without effect.

Figure 1 summarizes these results.

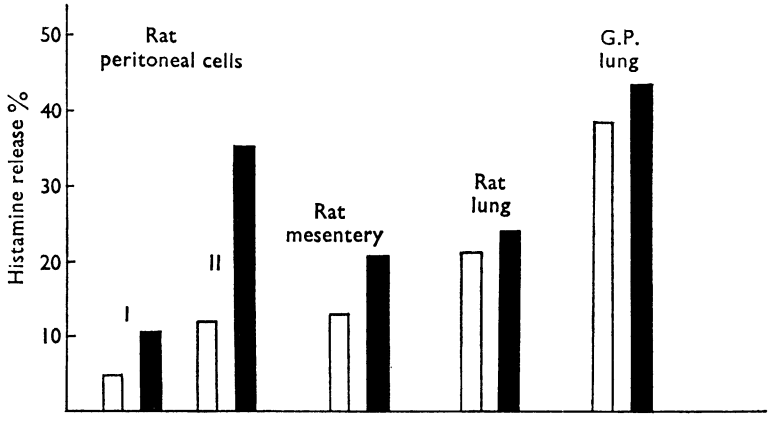

FIG. 1. Histamine release by antigen alone (open columns) and by antigen plus phosphatidyl serine (100 μg/ml) (filled columns) from various sensitized tissues. Two alternative sensitization schedules, I or II were used for the peritoneal cells.

(d) *Guinea-pig lung.* In 11 experiments with the chopped lung of sensitized animals PS increased antigen-induced release but the effect was marginal: a mean release of 40% increasing to 46%. The difference was not statistically significant ($>$0·05). The potentiation was 1·11. The question arises whether this small effect is due to releasing under conditions which already give nearly maximal release, since one seldom gets more than 50% release from chopped guinea-pig lung, even under optimal conditions. Experiments were therefore done with varying antigen concentrations to test this point. In the first series of tests the histamine release fell as the antigen concentration was reduced from 100 to 1 μg/ml but there was no greater potentiation. It was actually greatest with the highest antigen dose. In the second series of tests done on less well sensitized animals and with threshold concentrations of antigen (down to 0·05 μg/ml) we still obtained only marginal effects—never more than 10% increase due to the presence of the PS (Fig. 2).

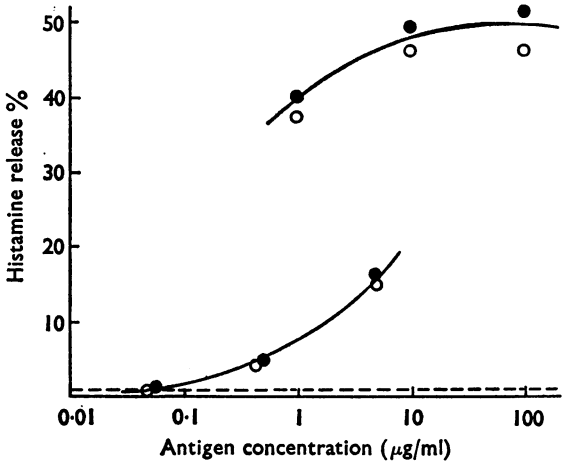

FIG. 2. Concentration-response curves for antigen alone (open circles) and antigen plus 100 μg/ml phosphatidyl serine (filled circles) releasing from sensitized guinea-pig lung tissue. There is no evidence for a larger effect of phosphatidyl serine when sub-maximal releases were produced.

Mechanism of potentiation by phosphatidyl serine

(a) *Time course.* The anaphylactic histamine release from isolated peritoneal cells has been found to be a very fast process; it was nearly complete 1 min after addition of antigen and after 5 min no further release could be detected (Fig. 3). As a measure of the initial rate of release we have used the 1 min release expressed as a fraction of the 5 min release. This ratio was 0·99 (mean of 4 experiments). The effect of PS on the time course of the release process was studied in 5 experiments. It increased the initial rate of release and slightly prolonged the release

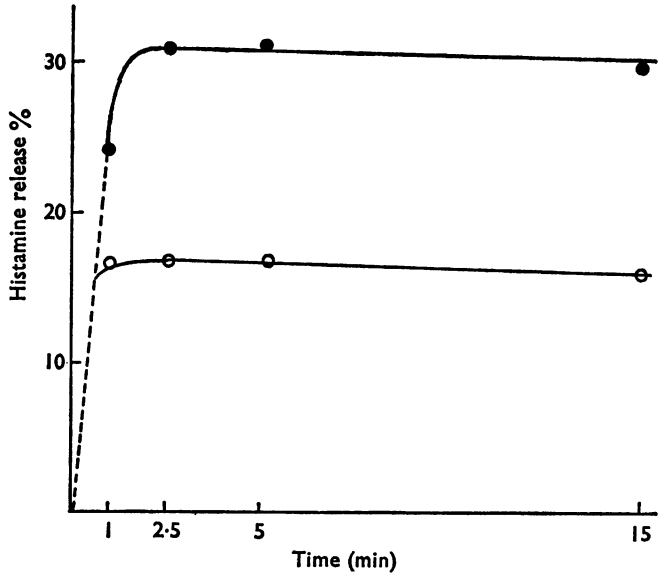

FIG. 3. Rate of histamine release from rat peritoneal cells by antigen alone (open circles) and antigen plus 100 μg/ml phosphatidyl serine (filled circles).

process. Thus, PS nearly doubled the first minute release but the 1 to 5 min ratio was still nearly unity (0·87).

With mesentery the 1 to 5 min ratio with antigen alone was 1·00. The stimulus to the initial rate of release by PS was not apparent in this tissue: PS increased the 1 min release by only 24% and the 1 to 5 min ratio was 0·80.

No pre-incubation of the cells with the phospholipid was necessary for optimal effects. On the contrary, PS was slightly more effective when added with the antigen than when added 5 min before the antigen: the potentiation was increased from 1·76 to 2·01 for 1 min releases and from 1·91 to 2·06 for 5 min releases (mean of 5 experiments, 3 with peritoneal cells and 2 with mesentery). These differences are not statistically significant ($P=0·1$).

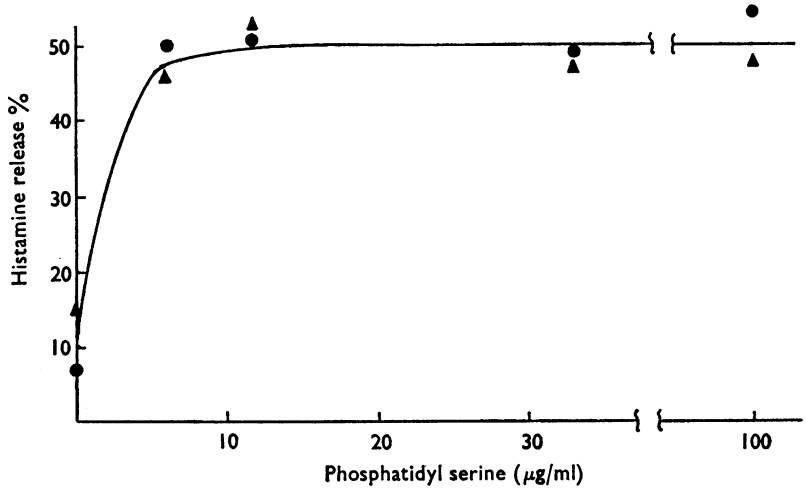

FIG. 4. Concentration response curve for release from rat peritoneal cells by antigen alone and in various concentrations of phosphatidyl serine (2 experiments).

(b) *Concentration of phosphatidyl serine.* Most of our experiments were done with 100 μg/ml of PS as a suspension in Tyrode. This concentration produced little histamine release above the spontaneous level and did not interfere with bioassays. The concentration–response curve, however, showed that as little as 10 μg/ml of PS produced a maximum effect (Fig. 4). The potentiation was constant over the range 10 to 100 μg/ml of PS ; there was no indication of a depression of the effect at high concentrations.

In a further series of experiments lower concentrations of 0·75, 1·5 and 3 μg/ml were used. A concentration-dependent potentiation was obtained for the two higher concentrations (Table 2).

(c) *Antigen concenration-response curve.* Most of the experiments described were done with supra-maximal concentrations of antigen (100–500 μg/ml). The effect of PS on release by antigen in the range 0·03 to 3 μg/ml was also tested. The results illustrated in Fig. 5 show that the phosphatidyl serine effect is obtained at all antigen concentrations though the potentiation ratio is especially high with low releases. In separate experiments the potentiation ratio was constant for antigen concentrations in the range 5–500 μg/ml.

TABLE 2. *(a) Histamine release from rat peritoneal cells by antigen alone and in the presence of threshold concentrations of phosphatidyl serine. Maximum effects with 50 μg/ml were also determined in 4 of the 6 experiments. Spontaneous releases have been subtracted. (b) Potentiation calculated from data in Table 2 (a).*

Expt. No.	1	2	3	4	5	6	
PS Conc. (μg/ml)			*(a) Histamine release*				
0	10	32	7	14	33	6	
0·75	9	39	8	20	33	10	
1·5	14	53	8	24	43	9	
3	12	59	10	31	39	8	
50	—	74	18	—	64	40	
			(b) Potentiation				Mean ± S.E.M.
0·75	0·9	1·2	1·1	1·4	1·0	1·7	1·23 ± 0·12
1·5	1·4	1·7	1·1	1·7	1·3	1·5	1·45 ± 0·10
3	1·2	1·8	1·4	2·2	1·2	1·3	1·53 ± 0·17
50	—	2·5	2·6	—	1·9	6·7	3·43 ± 1·10

(d) *Specificity:* In addition to PS, other phospholipids were tested: phosphatidyl inositol, choline ethanolamine and phosphatidic acid. None was appreciably active.

(e) *Effect of washing cells.* The experiments described above show that antigen-induced release of histamine from isolated mast cells in the peritoneal washings was readily potentiated by PS whilst that from mast cells in lung tissue was only slightly affected; the response of mast cells in mesentery was intermediate. It seemed likely that potentiations produced by phospholipid were the result of replacing some membrane constituent lost from isolated cells either *in vivo* or during the preparation of the cells for the histamine release process.

The peritoneal washings are normally spun down, resuspended in Tyrode and antigen then added. In a series of 4 experiments this procedure was modified so that antigen was added either directly to the peritoneal washings or to cells that had been washed 1, 2 or 3 times. The results are presented in Table 3. They provided very little support for the hypothesis that a cell constituent essential for the release process is lost by washing. In experiment 1 the release by antigen

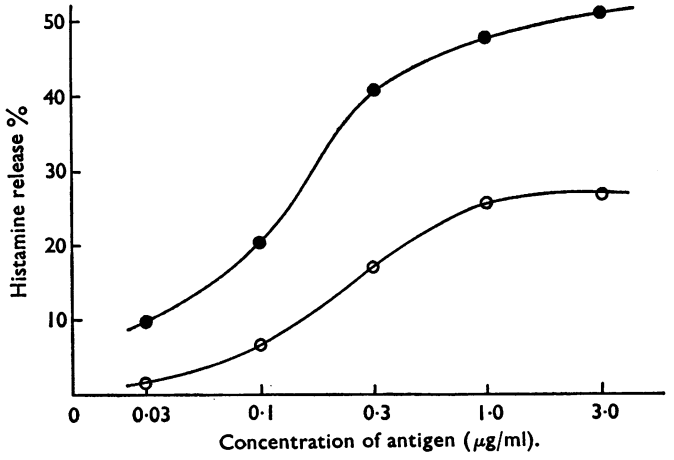

FIG. 5 Concentration-response curve for release by various concentrations of antigen alone (open circles) and antigen plus 10 μg/ml phosphatidyl serine (filled circles). Means from 3 experiments.

TABLE 3. *Effect of washing cells on histamine release by antigen (Ag) (100 μg/ml) and potentiation of release of phosphatidyl serine (PS) (10 μg/ml). Release (% of cell content) corrected for spontaneous release*

Washes	(Normal procedure) 0			1			2			3		
Expt.	Ag	Ag + PS	Pot^n	Ag	Ag + PS	Pot^n	Ag	Ag + PS	Pot^n	Ag	Ag + PS	Pot^n
1	7	50	6·7	27	79	2·9	—	—	—	—	—	—
2	12	57	5·0	13	74	5·9	18	79	4·4	—	—	—
3	—	—	—	15	36	2·5	11	31	2·8	8	25	3·3
4	—	—	—	52	81	1·6	42	80	1·9	37	77	2·1

plus PS increased from 50 to 79% as a result of washing, but the potentiation decreased from 6·7 to 2·9; the difference can largely be accounted for by the higher release with antigen alone from washed cells than from the unwashed suspension. In experiment 2 a second wash was introduced but did not result in any further potentiation. In 2 more experiments the effect of repeated washing was determined. There was a general decline in the absolute amount of histamine released and only a small increase in potentiation.

(f) *Cooperative action of cells.* There was no evidence that any cell types other than the histamine-containing mast cells were required in the potentiation by PS. In 3 experiments mast cells were concentrated by fractionating peritoneal washings over a bovine serum albumen gradient. They gave potentiations of 0·1, 3·1 and 5·1 with 100 μg/ml PS.

(g) *Role of calcium.* The spontaneous release of histamine from peritoneal cells suspended in Tyrode solution is independent of calcium ions in the range of 0–10 mM Ca^{2+} (Fig. 6). This is also true of cells suspended in Tyrode containing 100 μg/ml PS (Foreman & Mongar, to be published). The release by antigen, however, with or without PS, does depend on Ca ions. For antigen alone the slope

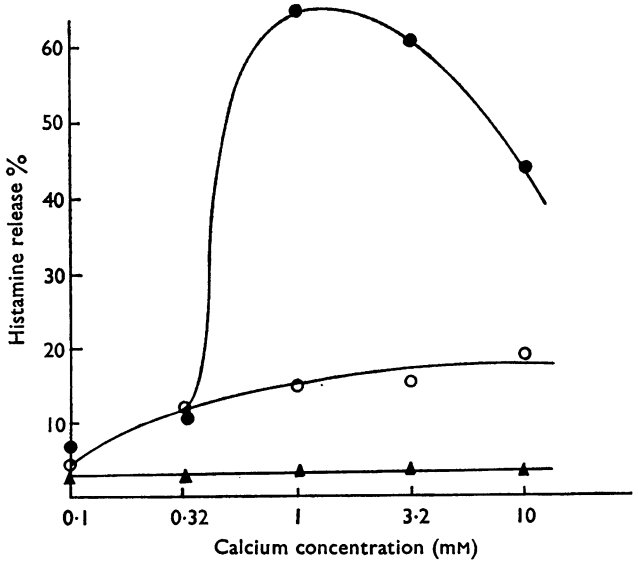

FIG. 6. Effect of calcium ion concentration on potentiation by phosphatidyl serine (10 μg/ml) of histamine release: spontaneous release ▲; release by antigen alone ○; release by antigen plus phosphatidyl serine ●.

is rather flat (Foreman & Mongar, 1972) but in the presence of 100 μg/ml PS a very steep slope is obtained (Table 4) resulting in a large potentiation (nearly 6 fold) due to the combined effect of 10 mM Ca and 100 μg/ml PS. In 2 of the 4 experiments of Table 4 this 'super potentiation' was already apparent in 1 mM Ca and in the experiment shown in Fig. 6 maximum potentiation was obtained with 1 mM Ca and 10 μg/ml PS.

TABLE 4. *Effect of various calcium concentrations on histamine release produced by phosphatidyl serine (PS) (100 μg/ml) and antigen (Ag) (100 μg/ml)*

(Ca^{++}) mM	PS	Ag	Experiment No. 1	2	3	4	Mean
1·0	−	−	8·5	6	3	4	5·4
„	+	−	11·5	7	3	9	7·6
0·01	−	+	8·5	8·5	5	5·5	6·9
„	+	+	9	9·5	4	24·5	11·9
0·1	−	+	8·5	8	4·5	5	6·5
„	+	+	14	12·5	8·5	24·5	15·0
1·0	−	+	14·5	10	8	4·5	9·3
„	+	+	15	15	20	25·5	19·0
10·0	−	+	11·5	12	8	4·5	9·0
„	+	+	33	20·5	32	29·5	28·8

The small effects of PS on the anaphylactic histamine release from intact mesentery or chopped lung in normal Tyrode solution (1·8 mM Ca) or Tyrode containing 1 mM Ca could not be increased by raising the calcium concentration to 10 mM. On the contrary 10 mM Ca abolishes the PS potentiation in these tissues. In two experiments with rat mesentery potentiation with 1 mM Ca was 2·2 but with 10 mM Ca it was only 0·8. A similar result was obtained in a single experiment with rat lung: potentiation fell from 1·25 to 1·06 due to the raised Ca concentration.

Discussion

Phosphatidyl serine augments the anaphylactic release of histamine. In rat tissues the effect was greatest for isolated peritoneal cells which gave a potentiation of 2·3 to 2·9 compared with 1·6 for mesentery and 1·2 for lung. Guinea-pig lung showed almost no potentiation (1·1) even when the antigen concentration was reduced to threshold values. The other phospholipids studied, phosphatidyl inositol, ethanolamine and choline were inactive.

Phospholipids were first shown to affect histamine release by Goth (1966) who used a lipid extract of brain called 'thromboplastin'. This greatly increased histamine release by dextran and ovomucoid from the peritoneal mast cells of certain strains of rats. PS was specifically implicated (Goth & Adams, 1970).

A number of other compounds have been found to augment the anaphylactic release of histamine from isolated tissues: succinate (Moussatche & Danon, 1957), thiols and denaturing agents (Edman, Mongar & Schild, 1964) raised temperature (Mongar & Schild, 1957) and pH (Mongar & Schild, 1958) but no definite site of action has been identified. Unlike the action of thiols and denaturing agents, potentiation is not succeeded by antagonism as concentration of the agent is increased. In the case of PS it seems likely that this compound acts by replacing or substituting for some constituent lacking from isolated cells. Lichtenstein (1968) produced an 'extract' from normal human leucocytes that enhanced the release of antigen from sensitized cells, especially when they had been repeatedly washed.

The factor was non-dialyzable and heat stable. Our experiments with cells that had been given additional washings are not inconsistent with this replacement hypothesis, but the enhancement of potentiation was not large, 1·6, 1·9 and 2·1 for successive washings. Alternatively, the deficiency of phospholipid may already exist before the cells are washed at all. The initial peritoneal suspension responded as well to phospholipid as did once washed cells and washing only seems to increase the deficiency slightly.

It is noteworthy that PS potentiated histamine release in a wide range of conditions ; e.g. strongly sensitized cells were potentiated as well as or better than weakly sensitized ones. Further, release resulting from a powerful stimulus of a supramaximal dose of antigen was as effectively potentiated as that produced by a near-threshold dose of antigen provided, of course, that the cells did not approach complete depletion.

The action of PS was rapid. It produced as much, or slightly more, potentiation when added with the antigen as when added 5 min before. These results suggest that the PS does not have to penetrate very far before producing its action. It probably acts on the cell membrane. Measurements of histamine release for different times showed that with peritoneal cells, PS acted mainly by increasing the initial rapid anaphylactic release which occurs within 1 min of adding antigen ; the effect was not due to a prolongation of the release process.

The calcium requirements of the anaphylactic reaction have been previously described (Mongar & Schild, 1958 ; Foreman & Mongar, 1972). Like many other secretory systems (Douglas, 1968) the stimulus-secretion coupling is dependent on calcium ions. In chopped perfused guinea-pig lung (Mongar, 1970) histamine release increases when the calcium concentration is increased above the physiological values. In other cells such as the leucocytes of man (Osler, 1964) and of rabbits (Greaves & Mongar, 1968) it decreases. In the rat tissues we have studied there was no increase in the release with antigen alone when the calcium concentration was increased from 1 to 10 mM calcium, but the potentiating effect of PS was very sensitive to the Ca ion concentration and 5 fold potentiations could be obtained with optimal concentrations which varied from one cell preparation to another.

Phospholipid-calcium interactions may be of general importance ; the calcium contained in the cell is largely concentrated in the phospholipid-rich membrane fractions (Hodgkin & Keynes, 1957) and when these fractions are extracted by organic solvents the protein-containing residues do not bind calcium whereas the extracted lipids (mainly phospholipids) do (Koketsu, Kitamura & Tanaka, 1964). Our findings show that the acidic serine potentiates the anaphylactic reaction. It also binds calcium (Hauser & Dawson, 1967). The neutral ethanolamine and choline compounds do not potentiate anaphylactic histamine release nor do they bind calcium. The acidic inositol which binds calcium, and which we thought in our preliminary communication also potentiates, is in fact biologically inactive. In this respect it is anomalous.

The interaction of PS with calcium and other divalent cations is worth further study ; it may provide a clue to understanding the chain of events initiated by the stimulus of the union of antigen with cell-fixed antibody which leads to the secretion of the contents of the mast cell granules. The final stage in the secretory process is probably fusion of the plasma and granule membranes leading to exocytosis. It is possible that this process is initiated by the entry of Ca ions into the cell so

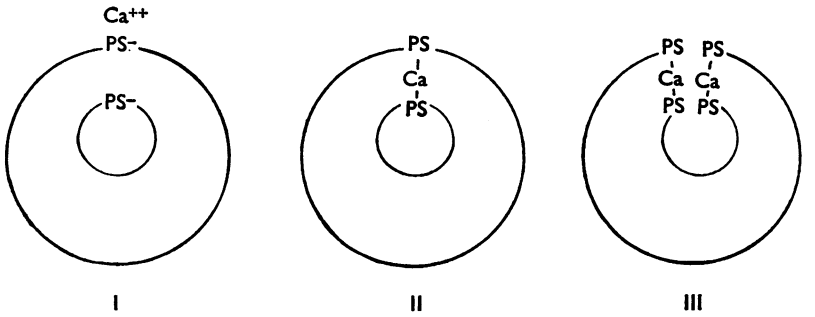

FIG. 7. Scheme for role of phosphatidyl serine (PS) and calcium ions in secretion of histamine from mast cells. I—resting state. II—stimulus leads to Ca^{++} entering the cell and bridging plasma and granule membranes. III—fusion of membranes leading to exocytosis.

that a link between the granule and cell membranes is formed by Ca-phosphatidyl serine electrostatic bridges, as depicted in Figure 7.

The availability of the phospholipid added to the suspension of mast cells has to be considered. The large proportion of lipid-soluble hydrocarbon in the molecule results in the formation of micelles and there is an equilibrium between these and 'dissolved' molecules. This limits the effective concentration and probably accounts for the flat dose response curve above a concentration of 10 μg/ml PS. A further limit on availability of acidic phospholipid added to cells may be set by formation of calcium phospholipid electrostatic bridges in the incubation fluid, for the calcium ions were usually present in about 100 times the equivalent concentration of phospholipid. However, the finding that one acidic phospholipid is active at a concentration of about 1 μg/ml whereas the neutral compounds are inactive at 100 μg/ml suggests that this is not a limiting factor determining their activity.

REFERENCES

DOUGLAS, W. W. (1968). Stimulus-secretion coupling: the concept and clues from chromaffin and other cells. (First Gaddam Memorial Lecture). *Br. J. Pharm. Chemother*, **34**, 451–474.

EDMAN, K. A. P., MONGAR, J. L. & SCHILD, H. O. (1964). The role of SH and S-S groups and oxygen in the anaphylactic reaction of chopped guinea-pig lung. *J. Physiol. (Lond.)*, **170**, 124–127.

FOREMAN, J. C. & MONGAR, J. L. (1972). The role of the alkaline earth ions in anaphylactic histamine secretion. *J. Physiol. (Lond.)*, **224**, 753–769.

GOTH, A. (1966). Mechanisms of histamine release by polymeric compounds. In: *Mechanisms of Release of Biogenic Amines*, ed. von Euler, U. S., Rosell, S. & Uvnas, B., Oxford: Pergamon.

GOTH, A. & ADAMS, H. R. (1970). Selective effect of phosphatidyl serine on macromolecular histamine release in the rat. *Fedn Proc.*, **69**, 2087.

GOTH, A., ADAMS, H. R. & KNOOHUIZEN, M. (1971). Phosphatidyl serine: selective enhancer of histamine release. *Science*, **173**, 1034–1035.

GREAVES, M. W. & MONGAR, J. L. (1968). The mechanism of anaphylactic histamine release from rabbit leucocytes. *Immunology*, **15**, 743–749.

HAUSER, H. & DAWSON, R. M. C. (1967). The binding of calcium at lipid-water interfaces. *Eur. J. Biochem.*, **1**, 61–69.

HODGKIN, A. L. & KEYNES, R. D. (1957). Movement of labelled calcium in squid giant axons. *J. Physiol (Lond.)*, **138**, 253–287.

KOKETSU, K., KITAMURA, R. & TANAKA, R. (1964). Binding of calcium ions to cell membranes isolated from bullfrog skeletal muscle. *Am. J. Physiol.*, **207**, 509–512.

LICHTENSTEIN, L. M. (1968). Mechanism of allergic histamine release from human leucocytes. In: *Biochemistry of the Acute Allergic Reactions*, ed. Austen, K. F. & Becker, E. L., pp. 153–174, Oxford: Blackwell.

LICHTENSTEIN, L. M. & OSLER, A. G. (1966). Studies on the mechanisms of hypersensitivity phenomena XI. The effect of normal human serum on the release of histamine from human leucocytes by ragweed pollen antigen. *J. Immunology*, **96**, 159–168.

MONGAR, J. L. (1970) In: *Calcium and Cellular Function*, ed. Cuthbert, A. W., pp. 280–283, London: Macmillan.

MONGAR, J. L. (1971). In: *Biochemistry of the Acute Allergic Reactions*, ed. Austen, K. F. & Becker, E. L., p. 88, Oxford: Blackwell.

MONGAR, J. L. & SCHILD, H. O. (1956). Effect of antigen and organic bases on intracellular histamine in guinea-pig lung. *J. Physiol. (Lond.)*, **131**, 207–219.

MONGAR, J. L. & SCHILD, H. O. (1957). Effect of temperature on the anaphylactic reaction. *J. Physiol. (Lond.)*, **135**, 320–338.

MONGAR, J. L. & SCHILD, H. O. (1958). The effect of calcium and pH on the anaphylactic reaction. *J. Physiol. (Lond.)*, **140**, 272–284.

MONGAR, J. L. & Svec, P. (1972). Effect of phospholipids on histamine release. *Br. J. Pharmac.*, **44**, 327P.

MOUSSATCHE, H. & DANON, A. P. (1957). On the action of succinate and malonate upon the histamine released during the anaphylactic reaction *in vitro*. *Naturwissenschaften*, **49**, 330.

OSLER, A. G. (1964). Studies of the mechanism of hypersensitivity phenomena: IX Histamine release from human leucocytes by Ragweed pollen antigen. *J. exp. Med.*, **120**, 507–530.

PERERA, B. A. V. & MONGAR, J. L. (1963). The role of a chymotrysin-like enzyme in histamine release from rat mast cells. *Immunology*, **6**, 472–477.

WHEELER, K. P. & WHITTAM, R. (1970). ATP-ase activity of the sodium pump needs phosphatidyl serine. *Nature (Lond.)* **225**, 449–450.

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