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HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS BY PHOSPHOLIPASE A. THE "ACTIVATION" OF PHOSPHOLIPASE A BY PHOSPHOLIPIDS

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Abstract—Phospholipase A did not release mast cell histamine when incubated with isolated rat mast cells in Tyrode solution. The phospholipid breakdown product lysolecithin (10–1000 $\mu g.ml^{-1}$) did release histamine from mast cells incubated in Tyrode solution. This release was not affected by changes in the incubation temperature. Phospholipase A (30.4 $mU.ml^{-1}$–30.4 $U.ml^{-1}$) did release mast cell histamine from isolated mast cells when 188 $\mu g.ml^{-1}$ phosphatidylserine or phosphatidylcholine were added to the incubation medium or when phospholipase A and mast cells were incubated in fresh plasma. In this case the release of histamine was dependent on the incubation temperature. Thus lysolecithin appears to release mast cell histamine by a surface active, cytolytic process whereas phospholipase A releases mast cell histamine by a direct enzymic attack on the mast cell.

Phospholipase A increases vascular permeability when injected into rat skin by a mechanism which appears to be mediated by the mast cell amines histamine and 5-hydroxytryptamine [1–4]. However, pure phospholipase A *in vitro* does not release histamine from isolated rat peritoneal mast cells [2, 5, 6]. It has been postulated that phospholipase A *in vivo* releases mast cell histamine by an indirect action involving the production of lysophosphatides which do release mast cell histamine *in vitro* [3, 7, 8]. Although lysophosphatides release mast cell histamine *in vitro*, this action is markedly inhibited in the presence of albumin. Greater concentrations of lysophosphatides are required to release histamine from mast cells incubated in plasma than in Tyrode solution [2]. Thus Keller [2] considered that the production of lysophosphatides *in vivo* probably did not contribute to mast cell histamine release by phospholipase A.

The experiments described below demonstrate that phospholipase A will only release histamine from mast cells incubated in Tyrode solution containing phospholipid, or when plasma is used as an incubation medium. Evidence is presented suggesting that phospholipase A releases histamine from mast cells by an enzymic action directed against the mast cell.

MATERIALS AND METHODS

Materials

Bee venom phospholipase A was purchased from Sigma Ltd. Samples of this preparation were found to elute as a single protein from gel filtration and ion-exchange columns and were thus judged to be sufficiently pure for these experiments [4].

Phosphatidylcholine, lysolecithin, arachidonic acid and glycerylphosphorylcholine were also purchased from Sigma Ltd. Glycerylphosphorylcholine was separated from its cadmium chloride complex by passage through a mixed bed ion-exchange column.

The prostaglandins PGE_1, PGE_2 and $PGF_{2\alpha}$ were kindly provided by Dr. J. Pike of the Upjohn Co, Kalamazoo, MI. Human serum albumin was purchased from Kabi Ltd. and compound 48/80 from Wellcome Ltd. Bovine plasma was prepared from fresh heparinised bovine blood obtained from a local slaughter house. Histamine acid phosphate was purchased from BDH Ltd., as was phosphatidylserine, which was supplied as a solution in chloroform. Before use the solvent, chloroform, was evaporated to dryness under a stream of nitrogen and the residue, Phosphatidylserine, suspended in Tyrode solution by sonication (Kelly Ultrasonics).

Methods

(1) *Isolation of mast cells.* Wistar rats were killed with ether and a small incision was made in the abdominal wall. 10 ml of phosphate buffered saline (pH 7.4) was injected into the peritoneal cavity through the incision. The incision was temporarily closed whilst the abdomen of the rat was gently massaged. The fluid in the peritoneal cavity was then withdrawn, placed in polypropylene centrifuge tubes and centrifuged at 150 *g* for 5 min. The resulting cell pellet was gently resuspended in a small volume of phosphate buffered saline. Mast cells were separated from other peritoneal cells by centrifugation on an albumin density gradient similar to that used by Chakravarty and Zeuthen [9]. 1.0 ml of a 20% solution of human serum albumin was layered over 1.0 ml of a 25% solution in a polypropylene centrifuge tube. The peritoneal cell suspension was layered over the albumin gradient and centrifuged at 150 *g* for 5 min. Mast cells were found at the bottom of the 25% layer. This zone, which contained 90–95 per cent mast cells, was removed and diluted with Tyrode solution to a final

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volume of 4.0 ml giving a suspension containing 1325 ± 64 cells. μl^{-1} ($n = 6$).

(2) *Incubation.* Aliquots of the mast cell suspension (0.2 ml) were incubated in 4.0 ml of Tyrode solution, or fresh bovine plasma, at 37° for 30 min. After incubation the mixture was centrifuged at 150 *g* for 5 min, the supernatant decanted and 4.0 ml of fresh Tyrode solution added to the cell pellet. The cell pellet was then boiled at 100° for 10 min to release residual histamine. The histamine content of both cell pellet and supernatant were measured by bioassay or atropinised guinea pig ileum. From this data the percentage of histamine released was calculated.

When cells were incubated with plasma or lysolecithin the percentage of histamine released was calculated by taking the histamine content of the control cell pellet as 100 per cent since plasma contains diamine oxidase, which metabolises any histamine released, and lysolecithin desensitises isolated tissue preparations [10].

In each experiment controls were included from which the spontaneous release of histamine from mast cells could be calculated and subtracted from experimental values. Experiments were not considered acceptable if spontaneous release exceeded 10 per cent. Similarly in each experiment mast cells were incubated with 25 $\mu g . ml^{-1}$ compound 48/80. In this case experiments were not considered acceptable if histamine release was less than 75 per cent.

RESULTS

(a) *Mast cell histamine release by phospholipase A.* Bee venom phospholipase A at concentrations up to 39 $U . ml^{-1}$ did not release histamine from mast cells incubated in Tyrode solution at 37° for 30 min. However, when phosphatidylserine (188 $\mu g . ml^{-1}$) was present with the enzyme a concentration dependent release of histamine was obtained (Fig. 1a). Phosphatidylcholine (188 $\mu g . ml^{-1}$) and the use of plasma as an incubation medium also produced an "activating" action (Figs 1b, c) although they appear

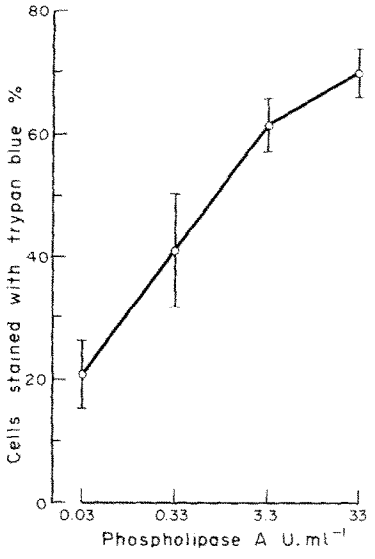

Fig. 2. The uptake of Trypan Blue by rat peritoneal mast cells following incubation in Tyrode solution containing Phospholipase A and Phosphatidylserine (188 $\mu g . ml^{-1}$) for 30 min at 37°. Phospholipase A and phosphatidylserine were incubated at 37° for 30 min before the addition of mast cells.

to release 20–30 per cent of the histamine by an action independent of phospholipase A.

Mast cell histamine release by phospholipase A and phosphatidylserine was associated with a loss of cell viability. Trypan blue exclusion studies revealed that the percentage of cells staining with trypan blue increased with the concentration of phospholipase A (Fig. 2). Thus it would appear that mast cell histamine release by phospholipase A and added phospholipids is brought about by a cytotoxic action probably involving destruction of the cell membrane by phospholipase A.

Lysolecithin, the product of phospholipid degradation by phospholipase A, also releases mast cell histamine in a concentration dependent manner.

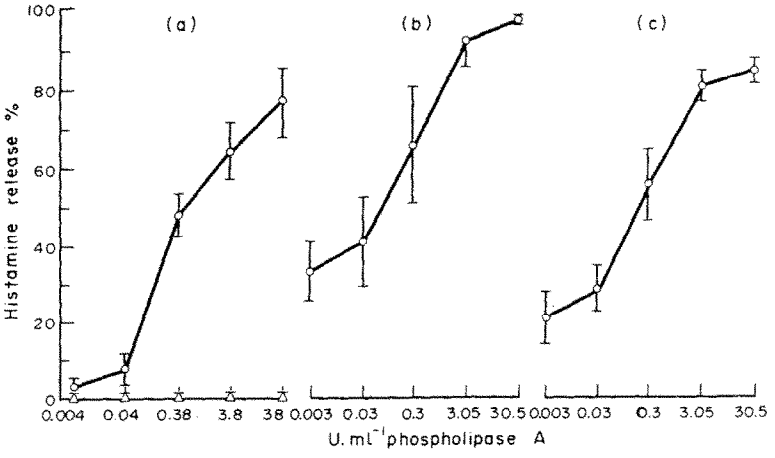

Fig. 1. The release of histamine from rat peritoneal mast cells by phospholipase A in (a) Tyrode (△—△) and Tyrode + Phosphatidylserine (188 $\mu g . ml^{-1}$) (○—○); (b) Tyrode + Phosphatidylcholine (188 $\mu g . ml^{-1}$), (c) Plasma. Phospholipids or plasma were incubated with phospholipase A for 30 min at 37° before the addition of mast cells which were then incubated at 37° for a further 30 min.

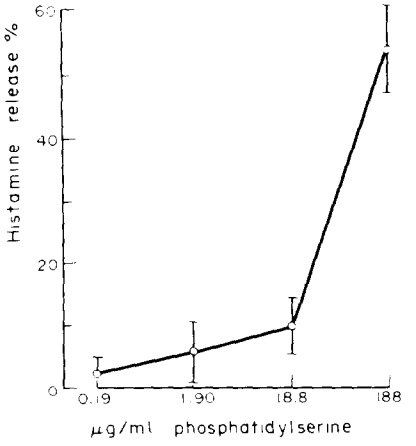

Fig. 3. Concentration effect curve for phosphatidylserine release of mast cell histamine in the presence of 3.9 U.ml^{-1} phospholipase A. Phospholipase A and phosphatidylserine were incubated at 37° for 30 min before the addition of mast cells which were then incubated at 37° for a further 30 min.

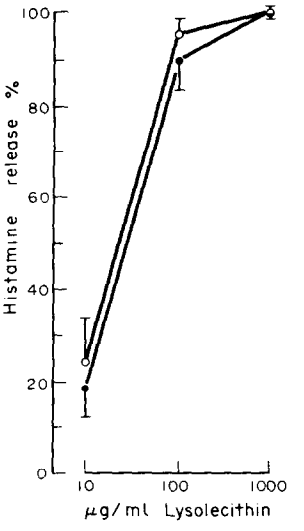

Fig. 5. The effect of temperature on mast cell histamine release by lysolecithin. (●—● 5°C; ○—○ 37°C.)

The release of mast cell histamine by phospholipase A and phosphatidylserine is dependent on the concentration of phosphatidylserine present (Fig. 3) but not dependent on the length of time the phospholipid and enzyme are incubated together before mast cells are added to the medium (Fig. 4).

Glycerylphosphorylcholine (62.5 μg.ml^{-1}), the product of further deacylation of lysolecithin, did not release histamine from isolated mast cells when incubated in Tyrode solution for 30 min at 37°. Prostaglandins E_1 and E_2 have been reported to release mast cell histamine *in vivo* [12] however PGE_1, PGE_2, $PGF_{2\alpha}$ and the prostaglandin precursor arachidonic acid all failed to release mast cell histamine when incubated with isolated mast cells for 30 min at 37° at concentrations up to 10 μg.ml^{-1}.

(b) *Experiments in which the temperature of incubation was altered.* Histamine is released from isolated mast cells by lysolecithin (10–1000 μg.ml^{-1}). This release is not affected when the cells are incubated with lysolecithin at 5° instead of 37° for 30 min (Fig. 5). However if phospholipase A and phosphatidylserine (188 μg.ml^{-1}) are incubated for 30 min at 37°, cooled to 5°, and then incubated with mast cells for a further 30 min; histamine release is totally abolished (Fig. 6a). Similarly when plasma is used as an incubating medium mast cell histamine release by phospholipase A at 5° is reduced to a level which is not related to the concentration of phospholipase A (Fig. 6c).

The "activation" of phospholipase A by phosphatidylcholine (188 μg.ml^{-1}) is also temperature dependent (Fig. 6b). However, following incubation with phospholipase A for 30 min at 37° and then at 5° for a further 30 min with mast cells histamine release is significantly reduced but not abolished (Fig. 6b). In this case some phospholipid has probably been converted to lysophospholipid accounting for the histamine release seen at 5°.

Therefore the release of histamine induced by

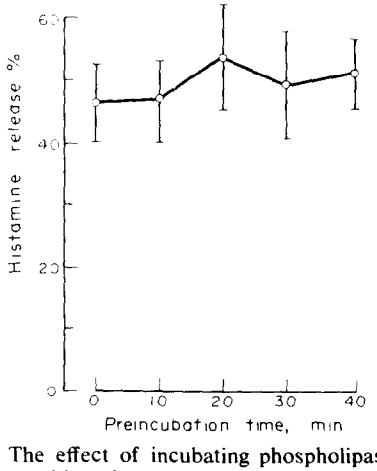

Fig. 4. The effect of incubating phospholipase A (3.05 U.ml^{-1}) with phosphatidylserine (188 μg.ml^{-1}) in Tyrode solution at 37° for different periods of time on the release of histamine from mast cells subsequently added and incubated at 37° for 30 min.

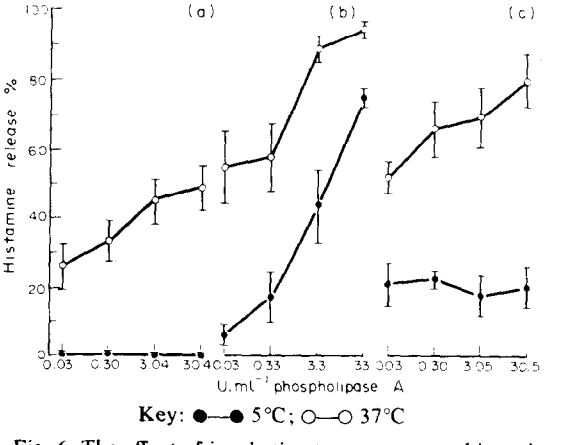

Fig. 6. The effect of incubation temperature on histamine release from mast cells by phospholipase A incubated in (a) Tyrode + Phosphatidylserine (188 μg.ml^{-1}), (b) Tyrode + Phosphatidylcholine (188 μg.ml^{-1}), (c) Plasma. Phospholipids or Plasma were incubated at 37° for 30 min before the incubation mixture was cooled to 5° and mast cells added. (●—● 5°C; ○—○ 37°C.)

lysolecithin is not dependent on the incubation temperature whereas that induced by phospholipase A in the presence of phospholipid or fresh plasma is significantly inhibited when incubated with isolated mast cells at 5°.

DISCUSSION

Phospholipase A and lysolecithin release mast cell histamine by two distinct mechanisms. In the case of lysolecithin, histamine release appears to be independent of incubation temperature. In contrast phospholipase A only releases mast cell histamine in the presence of phospholipid or when plasma is used as an incubation medium, and phospholipase A-induced mast cell histamine release is dependent on the temperature of incubation, even after the enzyme and phosphatide have been incubated for 30 min at 37°. Thus it would appear that phospholipase A induced mast cell histamine release is not mediated by extracellular lysophosphatides generated from the phospholipid in the medium by phospholipase A. Further evidence against a role for extracellular lysophosphatides in histamine release by phospholipase A can be found in the work of Keller who demonstrated that aqueous suspensions of phosphatides were not substrates for phospholipase A and that in plasma free lysolecithin is rapidly adsorbed onto plasma albumin [2].

Therefore it would appear that the added phospholipids have an "activating" action in the release of mast cell histamine by phospholipase A. The process of histamine release results in a loss of cell viability indicating that the release is probably the result of membrane breakdown by phospholipase A. Since histamine is not released from mast cells by phospholipase A in Tyrode solution alone the "activating" action of phospholipids is one which enables phospholipase A to gain access to the phospholipids of the cell membrane, alter the integrity of this structure and release histamine from the cell.

A mechanism involving an enzymic action is consistent with the temperature dependence of this process and at least three mechanisms of "activation" by phospholipids can be postulated. In the first the addition of phospholipid to the incubation mixture alters the configuration of the cell membrane such that the phospholipid core is exposed to phospholipase A and is thus enzymatically degraded. In the second case the added phospholipid binds to the cell and in doing so becomes a substrate for phospholipase A. The phospholipid could then be converted to the lysophosphatide, releasing cellular histamine without being released into the medium.

Data in the literature show that many enzymes are dependent on phospholipids for their activity. In the same way it could be postulated that phospholipase A will only utilise cell membrane phospholipids as a substrate when phospholipase A is present in a lipid–protein complex [13, 14]. However since most phospholipid dependent enzymes are integral parts of membranes, this third hypothesis is unlikely unless the added phospholipid and phospholipase A complex is bound to the cell becoming part of the cell membrane, which is then susceptible to attack by phospholipase A.

Phosphatidylserine has been shown to enhance mast cell histamine release induced by antigen and dextran [15]. This action occurs at much lower concentrations of phosphatidylserine than was used in the above experiments and is specific for phosphatidylserine. Thus it is unlikely that mast cell histamine release by phospholipase A and phospholipids has anything in common with the enhancing action of phosphatidylserine on mast cell histamine release by dextran or by antigen.

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