Short communications

Effect of calcium on dextran-induced histamine release from isolated mast cells

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Phosphatidyl serine potentiation of dextran-induced histamine release from isolated rat mast cells, was found to be calcium-dependent.

Introduction.—Goth & Adams (1970), and Goth, Adams & Knoohuizen (1971) have reported that histamine release induced by dextran from rat peritoneal mast cells was selectively potentiated by phosphatidyl serine. Mongar & Svec (1972) have shown that the anaphylactic histamine release from these cells was also potentiated by phosphatidyl serine, and that the degree of potentiation was dependent on calcium ions. It was of interest, therefore, to determine the effect of calcium on dextran-induced histamine release in the presence of phosphatidyl serine. The interaction of calcium with other phospholipids was also studied in this system.

Methods.—Lister Hooded, Sprague Dawley and Wistar strains of rat were used to compare the effects of dextran as a histamine releasing agent in the different strains. All the strains of rat were kept under identical conditions in the animal house.

The method of isolating the peritoneal mast cells and measuring histamine release from them has been described in an earlier paper (Foreman & Mongar, 1972). The medium used for incubating the cells was Tyrode solution and had the following composition (mM): 137·0 NaCl, 2·7 KCl, 5·6 glucose, 0·4 NaH_2PO_4, 1·0 $MgCl_2$, 1·8 $CaCl_2$, 12·0 $NaHCO_3$. All chemicals were of Analar quality and variations of the calcium concentration were achieved by adding appropriate amounts of calcium chloride stock solution (B.D.H. 1 M) to calcium-free Tyrode solution.

Phosphatidyl serine was supplied by Koch-Light Ltd., and was subjected to thin layer chromatography in chloroform-methanol-water. Impurities were identified when the plate was stained with ninhydrin and phosphomolybdic acid. When a known amount of phosphatidyl serine was hydrolyzed and subjected to amino acid analysis, traces of aspartic and glutamic acids and alanine, glycine and leucine were found, but the assay of serine was about 80%. Phosphatidyl inositol, kindly supplied by Dr. E. Lea, University of East Anglia, was the only one of five preparations of this compound which moved as a single spot on thin layer chromatography, when a 50 μg sample was applied to the plate. The choline and ethanolamine compounds were synthetic (Koch-Light Ltd.).

Dextran 110, used to release histamine, was supplied as a 6% solution in 0·9% saline (Fisons).

Histamine was assayed on the guinea-pig ileum and at the concentration used, the phospholipids did not affect the assay.

All values for histamine release have been corrected for the release in the absence of dextran, that is, spontaneous release.

Results.—In normal Tyrode solution, 6 mg/ml dextran produced a small release of histamine from the mast cells of all three strains of rat; the release being about 1% of the total cell histamine. Phosphatidyl serine potentiated the dextran-induced histamine release from all three strains of rat, but the choline and ethanolamine compounds were without effect. The results were qualitatively the same in the three strains, but the potentiation caused by 10 μg/ml phosphatidyl serine was about five fold for cells from Wistar rats whilst in Sprague Dawley and Lister Hooded rats the potentiation by the same concentration of phosphatidyl serine was greater than ten fold.

Dextran was found to release only a trace (<2%) of histamine from mast cells in the absence of calcium, and increasing the calcium concentration from zero to 1·0 mM produced only about a two fold increase in the release (Fig. 1). The potentiation of dextran-induced histamine release by 10 μg/ml phosphatidyl serine was found to be dependent on calcium. In the absence of calcium, phosphatidyl serine had no effect but as the concentration of calcium was raised in the range 0·1 to 1·0 mM, a graded increase of histamine release was observed, and at a

concentration of 1 mM calcium, the dextran-induced histamine release was increased by about ten fold or more in the presence of phosphatidyl serine. At higher concentrations of calcium, some depression of the potentiated histamine release by dextran was observed. In the presence of the choline, inositol and ethanolamine compounds and phosphatidic acid itself, at concentrations of 10 μg/ml, calcium had very little effect on the release when its concentration was varied in the range 0 to 10 mM (Fig. 1).

Discussion.—It is clear from the results that histamine release from isolated rat mast cells induced by dextran in the presence of phosphatidyl serine is dependent on calcium. The range of calcium concentrations over which a graded increase in histamine release was observed was 0·1 to 1·0 mM which is the same range of concentrations of calcium producing a graded effect on anaphylactic histamine release (Foreman & Mongar, 1972).

Histamine release by dextran is not totally dependent on calcium, since it will occur when the cells are suspended in calcium-free Tyrode or in physiological saline (0·9% w/v NaCl solution) (unpublished observation), and the release is enhanced by the presence of calcium but the effect is small compared with that obtained in the presence of phosphatidyl serine. Therefore, phosphatidyl serine acts in some way to facilitate the involvement of calcium in the release process, and this property is apparently specific to the serine compound since the choline, ethanolamine and inositol compounds, and phosphatidic acid itself, did not possess it. The fact that phosphatidyl serine binds calcium may be significant, but it is interesting that phosphatidic acid and the inositol compound bind calcium (Hauser & Dawson, 1967), but do not potentiate dextran-induced histamine release.

The depression of dextran-induced histamine release by higher concentrations of calcium is in agreement with the *in vivo* findings of Hannahoe (1972), and anaphylactic histamine release is also depressed at high calcium concentrations (Greaves & Mongar, 1968 ; Foreman & Mongar, 1972). The depressing effect of calcium on mast cells may be comparable to a similar effect at the neuromuscular junction, where high calcium concentrations disrupt the secretory process and cause aggregation of the vesicles (Heuser, Katz & Miledi, 1971).

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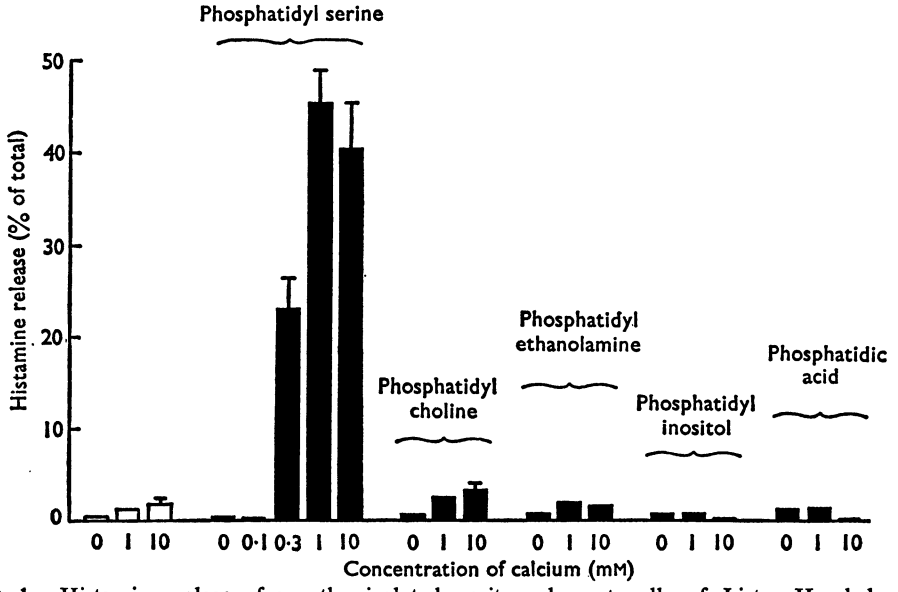

FIG. 1. Histamine release from the isolated peritoneal mast cells of Lister Hooded rats induced by 12 mg/ml dextran 110. Open columns are the histamine release by dextran alone. Solid columns are the histamine release by dextran in the presence of 10 μg/ml of phospholipid. Each column is the mean of duplicate samples and the vertical bars indicate the range.

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