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Histamine release from the mast cells of guinea-pig lung

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The distribution of mast cells and the effects of antigen on the mast cell population of sensitized guinea-pig lung have been examined. Doses of antigen, phospholipase A, trypsin, and compound 48/80 which released similar amounts of histamine also caused mast cell damage to similar extents following *in vitro* or *in vivo* administration. Pretreatment with ethanolamine, hydrocortisone or theophylline reduced the release of histamine and of SRS-A, and mast cell damage during subsequent anaphylaxis. Whilst there is evidence that the mast cell might be a selective target for the anaphylactic reaction in guinea-pig lung tissues, there is also evidence to suggest that the anaphylactic reaction induces generalized cell damage in these tissues.

IN 1956 Mota & Vugman reported that guinea-pig lung tissues had a high mast cell count and that sensitized animals given anaphylactic shock by the intracardiac injection of antigen exhibited a marked depletion of this mast cell population. Boreus & Chakravarty (1960) examined the *in vitro* effects of antigen on the mast cell content of guinea-pig lung tissues, and observed that the disappearance of mast cells could be correlated with the release of histamine and the slow reacting substance of anaphylaxis (SRS-A) from the tissue. These observations were extended by Boreus (1960, 1961) who made *in vivo* studies of the reactions of mast cells in the nasal mucosa of sensitized guinea-pigs to intra-arterial, intravenous, and topical administration of antigen.

Histamine can be released from sensitized guinea-pig lung tissue by trypsin, phospholipase A, and compound 48/80 as well as by antigen (Marquis & Smith, 1963). It is not known, however, whether the released histamine is in all instances derived from tissue mast cells. The histamine-releasing capacity of these substances has now been examined simultaneously with their effect on the mast cell population of guinea-pig lung tissues. The effect of pretreatment with anti-anaphylactic doses of theophylline (Firth & Smith, 1962), ethanolamine (Smith, 1961; Goadby & Smith, 1965), and hydrocortisone (Goadby & Smith, 1964; 1965) on the subsequent reaction of guinea-pig lung mast cells to anaphylaxis *in vitro* is also reported. These experiments were made to see if they supported the view advanced by Boreus & Chakravarty (1960) that the mast cell is the main, and perhaps the only, source of histamine and SRS-A released from guinea-pig lung tissue by antigen or histamine releasing agents.

Experimental

MATERIALS

Egg white was separated from the yolks of fresh eggs and freeze-dried immediately. The trypsin used was crystalline (B.D.H.). The Russell

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viper venom, used as a preparation of phospholipase A, and compound 48/80 were kindly donated by Messrs Burroughs Wellcome & Co.

INITIAL TREATMENT OF ANIMALS

Guinea-pigs of either sex, weighing between 200 and 250 g, were fed on Diet 18 pellets (Oxo Ltd.) and received 50 mg of ascorbic acid daily in their drinking water. Each animal was sensitized by the intraperitoneal injection of 100 mg of freeze-dried egg white dissolved in 2 ml of normal saline. After a sensitization period of 21 days, the animals were subjected to anaphylactic shock *in vivo* by the method of Herxheimer (1952) in which each animal is exposed to an aerosol of a 1% w/w solution of egg white in a Wright aerosolizer (Wright, 1958) at 15 lb/in.^{2} Animals were removed from the chamber in which they were exposed to antigen immediately before the characteristic convulsions of anaphylaxis. The time in seconds taken by each animal to reach this stage was termed the *collapse time* and was regarded as a measure of the sensitivity of each animal to the anaphylactic reaction. Seven to 10 days after exposure to the aerosol of antigen the animals were weighed and distributed into groups of six, such that the mean values for collapse time and body weight were approximately equal for each experimental group.

HISTOLOGY

Lung tissue was fixed according to Mota & Vugman (1956) by the injection of fixative through the trachea and main bronchi. The fixative solution contained 50% ethanol and 4% lead subacetate and was acidified with 0·5% glacial acetic acid. The tissue was stored in fixative for 24 hr before sections, 50μ thick, were cut routinely from samples (5 samples per lobe from 2 lobes per animal). These were cut on a freezing microtome and then stained with 0·1% ethanolic toluidine blue acidified to pH 4·0 with glacial acetic acid. Paraffin sections, 10μ thick, were also prepared for detailed histological examinations.

The frozen sections (2 sections/tissue sample; 20 sections/animal) were used to count the tissue mast cell population, according to Mota & Vugman (1956), Padawar (1963) and Boreus (1960). Each section was examined microscopically so that each field covered an area of 0·0154 mm^2 of lung tissue. Ten such fields were chosen at random for each section and the number of mast cells recorded. The total number in 100 fields was taken as the *mast cell count* (M.C.C.) for each animal. From these values a *mean mast cell count* (M.M.C.C.) for each group of animals was calculated. Differences in the values obtained for test and control groups were measured by the *mast cell disappearance value* (M.C.D.V.) introduced by Boreus (1960). This is given by:

$$\text{M.C.D.V.} = \frac{\text{M.M.C.C. (control)} - \text{M.M.C.C. (test)}}{\text{M.M.C.C. (control)}} \times 100$$

MEASUREMENT OF HISTAMINE RELEASE *in vitro*

Histamine released from perfused isolated lungs by the action of antigen, phospholipase A, cystalline trypsin, and compound 48/80 was collected by the method of Brocklehurst (1960). Except in the case of antigen, attempts to measure the histamine content of the vascular perfusates so obtained met with difficulty because contamination with histamine releaser interfered with the biological assay of histamine. To overcome this, before assay, perfusates containing histamine releasers other than antigen were extracted according to Barsoum & Gaddum (1935) as simplified by Code (1937). This treatment did not permit accurate assay of histamine in perfusates contaminated with compound 48/80, but the depression of histamine was consistent over the dose range of compound 48/80 used and was not great. A correction factor of 1·19 was calculated from data from control solutions of histamine containing added compound 48/80 and this factor was subsequently applied to all compound 48/80 perfusates. Four-point assays of histamine using a latin square design were made on guinea-pig terminal ileum in the presence of atropine 10^{-7}M. All values for histamine were calculated as histamine base.

ESTIMATION OF HISTAMINE RELEASE *in vivo*

Histamine release following the intracardiac injection of antigen, phospholipase A, trypsin, or compound 48/80 into groups of 6 animals was estimated by collecting 2 ml blood samples from each animal 5 min after injection. The samples from each group of animals were pooled into 20 ml of 10% aqueous trichloroacetic acid which was subjected to Code's extraction procedure. The extract was assayed for histamine content and the mean histamine value per ml of blood calculated. From the known body weights of the animals and the figures for blood volume per kg quoted by Dittmer (1961), the blood volume of each animal was obtained and the mean blood volume for each group of animals calculated. Using the mean blood volume and the estimated mean histamine content per ml, the total blood histamine of each group was calculated.

MEASUREMENT OF LUNG HISTAMINE CONTENT

The histamine content of guinea-pig lung tissues was determined by extraction from weighed samples of tissue after grinding with sand and 10% aqueous trichloroacetic acid solution in the proportions of 10 ml/g of wet tissue. This extract was treated by the procedure of Code (1937). The resulting aqueous solution of histamine was assayed for histamine base on guinea-pig terminal ileum in the presence of atropine 10^{-7}M. From the weights of sample and original whole lung, estimates were made of the total lung histamine content per animal.

MEASUREMENT OF THE SLOW-REACTING SUBSTANCE OF ANAPHYLAXIS

SRS-A was assayed on guinea-pig terminal ileum in the presence of atropine 10^{-7}M and mepyramine 10^{-7}M by comparison with a laboratory standard. In each case, a four-point assay of latin square design was made.

TREATMENT OF ANIMALS WITH ANTI-ANAPHYLACTIC AGENTS

Ethanolamine. Each animal received the equivalent of 200 mg/kg ethanolamine base as the hydrochloride each day for three days by intramuscular injection, the last injection being made 1 hr before death (Smith, 1961).

Hydrocortisone. Each animal was given a single intramuscular injection of 100 mg/kg of hydrocortisone (as the sodium hemisuccinate salt dissolved in water) 18 hr before death (Goadby & Smith, 1964).

Theophylline. A single dose of 80 mg/kg of theophylline (dissolved in water with the aid of ethylenediamine) was given intraperitoneally to each animal 15 min before death (Firth & Smith, 1962).

Results

THE DISTRIBUTION OF MAST CELLS IN GUINEA-PIG LUNG TISSUES

Tissue mast cells were observed to be widely distributed throughout the lung tissues. The cells were spherical or ovoid, between 10 and 25 μ in diameter or length, densely packed with granules which often obscured the nucleus and stained metachromatically. They were especially numerous in the pleura, in pleural invaginations into the parenchyma of the lung, and in the connective tissue surrounding the bronchi and bronchioles. They were much less numerous in the alveolar walls and the mucosa of the large bronchi.

THE RELEASE OF HISTAMINE *in vitro*

Histamine release from perfused sensitized guinea-pig lung tissues

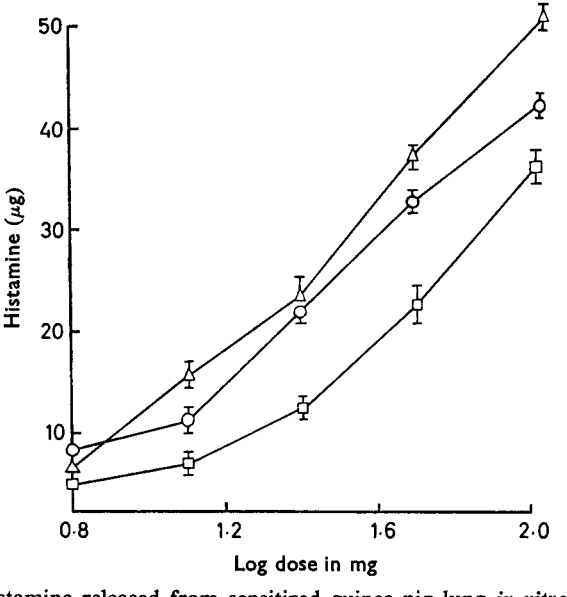

FIG. 1. Histamine released from sensitized guinea-pig lung *in vitro* by increasing doses of antigen ○, phospholipase A □, and trypsin △. Ranges indicated are standard errors.

induced by increasing doses of all four histamine releasers was measured. The results were plotted as log dose-effect curves. (Fig. 1). With antigen, phospholipase A or trypsin, increasing doses of each substance released increasing amounts of histamine. The curve obtained with compound 48/80 (Fig. 2) differed in that, as the dose was increased the

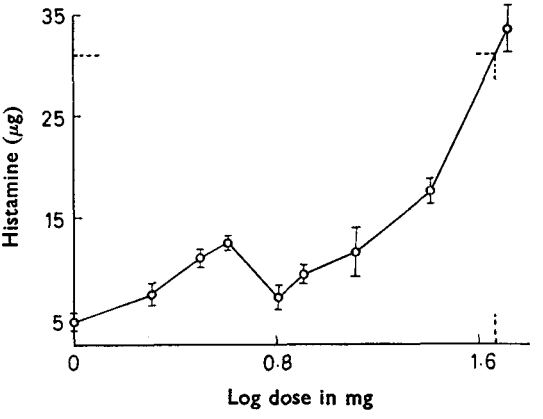

FIG. 2. Histamine released from sensitized guinea-pig lung *in vitro* by increasing doses of compound 48/80. Ranges indicated are standard errors.

release of histamine increased, then decreased, and finally increased again. This *dosage peak* was also examined in relation to mast cell disappearance.

MAST CELL DISAPPEARANCE *in vitro* AND *in vivo*

The mean mast cell counts for groups of animals exposed to increasing doses of antigen decreased. This is shown in Fig. 3 where the effect on the tissue mast cell population is expressed as the mast cell disappearance

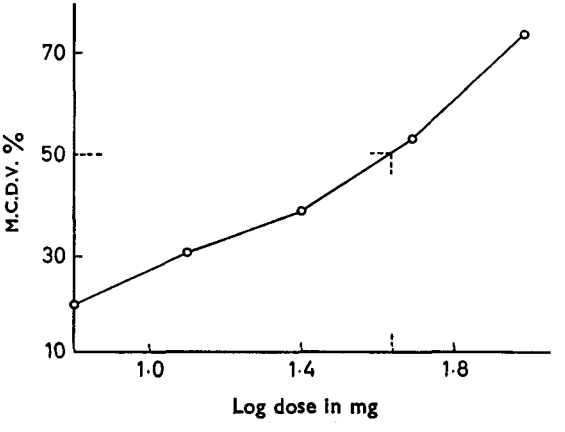

FIG. 3. Effect of increasing doses of antigen on the *mast cell disappearance value* (M.C.D.V.) observed in sensitized guinea-pig lung.

value. This value increases progressively with increasing doses of antigen. Apart from the disappearance of mast cells after anaphylaxis, some of the remaining mast cells were damaged but not totally degranulated, and there were also signs of peribronchial oedema and emphysema in the tissue.

It was found from Fig. 3 that 44 mg of antigen caused the disappearance of 50% of the mast cell population after anaphylaxis. From Fig. 1 it was estimated that this dose of antigen liberated 31 μg of histamine. From this figure it can be calculated that 31 μg of histamine was released by 35 mg of phospholipase A, or 77 mg of trypsin, or 45 mg of compound 48/80. These equiactive doses were then tested *in vitro* and *in vivo* to compare both their histamine-releasing properties and their effects on the mast cell population of guinea-pig lung tissue (Table 1).

TABLE 1. HISTAMINE RELEASE AND MAST CELL COUNTS OBSERVED AFTER THE ADMINISTRATION OF EQUIACTIVE DOSES OF HISTAMINE RELEASERS *in vitro* TO LUNGS REMOVED FROM GROUPS OF 6 SENSITIZED GUINEA-PIGS OR *in vivo* TO GROUPS OF 6 SENSITIZED GUINEA-PIGS

	Control	Antigen 44 mg	Phospholipase A 35 mg	Trypsin 77 mg	Compound 48/80 45 mg
In vitro					
Mean histamine release µg	0·35	30·7 (5·6)	33·4 (6·0)	28·8 (3·9)	26·1 (4·3)
Mean mast cell count	206·9 (34·2)	99·5 (19·6)	94·2 (16·3)	117·7 (16·9)	110·8 (1·6)
Mast cell disappearance value %	—	51·9	54·5	43·1	46·4
In vivo					
Estimates of mean circulating blood histamine µg ..	1·9	25·2	28·1	20·8	25·9
Mean mast cell count	206·9 (34·2)	110·2 (31·4)	99·5 (21·9)	111·0 (26·8)	120·8 (20·0)
Mast cell disappearance value %	—	46·7	51·9	46·3	41·6

Figures in parentheses are standard deviations.

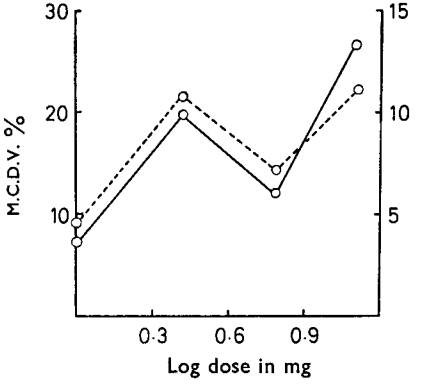

FIG. 4. Mast cell disappearance (O——O) and histamine release (O——O) in guinea-pig lung following increasing dosage with compound 48/80.

In each instance the histamine releasers effected a similar and substantial release of histamine; at the same time there was a large reduction in the numbers of mast cells in the tissue. Using Student's *t* test, it was found that histamine release and mast cell disappearance after equiactive doses of phospholipase A, trypsin, and compound 48/80 were not statistically significantly different from those induced by antigen.

Mast cell counts were also made on tissue removed from animals treated with small doses of compound 48/80 over the range in which an *initial low dosage peak* of activity had been observed. Fig. 4 shows that histamine release and mast cell disappearance followed similar courses over this dose range.

MAST CELL DISAPPEARANCE IN ANIMALS PRETREATED WITH ANTI-ANAPHYLACTIC AGENTS

Treatment of sensitized animals with ethanolamine or theophylline did not alter the lung histamine content or the mast cell population. Animals treated with hydrocortisone, however, differed from untreated animals in that lung histamine levels were significantly lower (at $P = 0.95$) and the mast cell count had the lowest mean value of any recorded in these experiments although it was not statistically different from the control value (Table 2). The effects of these three compounds on a

TABLE 2. THE EFFECT OF ANTI-ANAPHYLACTIC AGENTS ON THE HISTAMINE CONTENT AND MAST CELL COUNT OF LUNGS FROM GROUPS OF 6 SENSITIZED GUINEA-PIGS

Treatment	Total histamine content μg	Mean mast cell count
Nil	82·3 (10·3)	209·5 (38·2)
Ethanolamine	88·5 (20·4)	217·5 (39·8)
Hydrocortisone	69·8* (3·2)	184·0 (26·7)
Theophylline	73·3 (9·5)	201·0 (24·6)

Figures in parentheses are standard deviations.

* Difference from control is significant at $P = 0.95$.

subsequent anaphylactic reaction *in vitro* were similar (Table 3). Histamine and SRS-A release were reduced. At the same time the shock-induced fall in mast cell count was significantly reduced by all three anti-anaphylactic agents.

TABLE 3. THE EFFECT OF ANTI-ANAPHYLACTIC AGENTS ON THE RELEASE OF HISTAMINE, THE RELEASE OF SRS-A, AND THE MEAN MAST CELL COUNTS OF LUNGS TAKEN FROM GROUPS OF 6 GUINEA-PIGS AND SUBJECTED TO ANAPHYLAXIS *in vitro*

Treatment	Histamine release μg	SRS-A Release units/ml	Mean mast cell count
Nil	33·4 (4·7)	24·5 (9·3)	95·5 (17·9'
Ethanolamine	21·5* (4·8)	14·8* (4·3)	125·8* (18·6)
Hydrocortisone	16·2* (9·4)	14·3* (9·4)	131·2* (26·2)
Theophylline	20·2* (5·2)	14·7* (8·1)	121·2* (23·7)

Figures in parentheses are standard deviations.

* Difference from control is significant at $P = 0.95$.

Discussion

Doses of phospholipase A, trypsin and compound 48/80 causing the release of similar amounts of histamine from guinea-pig lung tissue either *in vitro* or *in vivo*, did not differ significantly in their ability to cause mast cell disappearance. In groups of guinea-pigs pretreated with anti-anaphylactic agents, anaphylactic shock *in vitro* resulted in the release of reduced amounts of histamine and SRS-A and a reduction in the fall in mast cell count. Collectively, these results support the hypothesis advanced by Boreus & Chakravarty (1960) that the mast cell is the main source of histamine and SRS-A released from guinea-pig lung by anaphylaxis or histamine-releasing agents.

From this evidence it might be argued that the mast cell in guinea-pig lung tissue is a selective target for the antigen-antibody reaction of anaphylaxis or histamine-releasing agents. However, equi-active doses of these agents do not produce identical effects on the lipid content of the tissue (Marquis & Smith, 1963). Phospholipase A and compound 48/80, like antigen, provoke a loss of phospholipid, but trypsin does not. Whereas antigen induces a fall in lung cholesterol, the other three agents do not have this effect. It therefore appears that the loss of lipid which occurs during histamine release is not confined to mast cells, but must be regarded as an index of more widespread cell damage.

Mast cells are fragile. They can be readily degranulated in mesentery if tension is applied to this tissue when spreading it on microscope slides. In view of this, the occurrence of mast cell degranulation in a tissue as the result of biochemical injury is a reasonable supposition. Other evidence is being accumulated in favour of a concept whereby anaphylaxis produces non-specific cell damage, rather than specific damage to mast cells. Guinea-pig lung subjected to anaphylaxis rapidly resynthesizes its lost phospholipid (Goadby & Smith, 1966). This recovery occurs in a few hours, and is unlikely to be a function of a mast cell population which requires at least 3 weeks for restoration to normal levels.

The demonstration of Riley & West (1953) that histamine is found in tissue mast cells has been repeatedly confirmed, and the present findings indicate that mast cell histamine is the source of histamine released in anaphylaxis. The same might be true of SRS-A. Whilst mast cells might also be the source of SRS-A, evidence for its occurrence in them is lacking; and although it is easy to assume that like histamine, SRS-A is released only from mast cell sources during anaphylaxis, the data so far obtained do not preclude the possibility that SRS-A is a product of more widespread cell damage. The histamine releasers examined also release a slow reacting substance (Marquis & Smith, 1963) but not the same substance for each releaser; a situation more compatible with widespread cell damage than with specific damage to one particular type of cell.

Thus it seems that an anaphylactic reaction in guinea-pig lung tissue induces two effects; a generalized cell damage involving loss of lipid from the tissue, and a disappearance of mast cells. If the latter effect

is dependent on the former, ethanolamine, hydrocortisone, and theophylline could be considered to exert their anti-anaphylactic effect by reducing the severity of the generalized cell damage and this would be manifest from one aspect as a reduction in mast cell disappearance.

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