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Induction of Histamine Release *in Vitro* from Rat Peritoneal Mast Cells by Extracts of Grain Dust

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The ability of extracts of grain dust and wheat to induce histamine release from rat peritoneal cells was investigated. Some grain dusts, with a high endotoxin content, were found to produce cytotoxic histamine release. Extract of wheat dust, with a low endotoxin release, produced noncytotoxic histamine release from peritoneal cells but not from purified mast cells. This reaction was dependent on the presence of phosphatidyl serine. The agent did not appear to be a lectin because histamine release was not enhanced by passive sensitization of mast cells with IgE. The activity occurred only over a narrow range of concentrations of the extract of wheat. The cause was unclear.

It is now generally accepted that people exposed to grain dust develop both acute and chronic respiratory disorders, including cough and phlegm, dyspnea, and airflow limitations (*1–5*). The mechanism(s) by which grain dust, or one of its constituents, produces its effects is unknown, although several possible mechanisms have been proposed.

Grain—either itself or in the form of flour—and contaminants of grain such as plant pollens, molds, or mites are all well-known inducers of IgE-mediated hypersensitivity reactions (*6*). Acute airflow obstruction may result from this mechanism in some grain workers, but, surprisingly not in a high proportion (*7*). The antigens present in grain dust could also induce the formation of specific antibodies of other immunoglobulin classes; subsequent antigen-antibody reactions might produce respiratory disorders. For example, Dutkiewicz has reported hypersensitivity pneumonitis in grain workers with precipitating antibodies to the bacterium *Erwinia herbicola* found in grain dust (*8*). Alternatively nonspecific precipitation of immunoglobulins can occur with grain dust and could produce inflammation (*9*).

Mechanisms not related to antibody formation have also been postulated. Olenchock and his colleagues have shown *in vitro* the activation of the complement system by various grain dusts; this mechanism has not yet been demonstrated *in vivo* (*10,11*). Endotoxins derived from the bacteria in grain dust could be a cause of inflammation in the lungs, by analogy with their possible involvement in byssinosis (*12*). This possibility has been investigated by Dutkiewicz et al., who studied an epidemic of acute respiratory disease in students exposed to grain (*13*). Finally, it has been suggested that grain dust might contain endogenous histamine or substances capable of inducing histamine release. The former possibility was investigated by Nicholls, who found that the concentration of histamine found in cereal grain dust was very low and hence unlikely to be capable of causing respiratory distress (*14*). Constituents capable of releasing histamine have been found in cotton dust by several investigators (*15–18*), and the purpose of the present study was to examine the possibility that grain dust might exert some of its adverse effects via a similar mechanism. We chose an *in vitro* system using rat peritoneal mast cells, since this was a well established model for the study of histamine release from mast cells. We report here the results of investigations of the capacity of grain dust and grain extracts to induce histamine release from these cells.

Materials and Methods

Various grain dusts were used in these studies. Grain dust had been collected from several Manitoba elevators and had been shown to produce acute airflow obstruction and grain feverlike symptoms on inhalation challenge in previous studies. These grain dusts, together with freshly harvested wheat, Manitoba Number 1 grade Sperling spring wheat, were used to prepare the grain dust extracts. The dust and the whole wheat were extracted in Coca's solution (86 mM NaCl, 33 mM $NaHCO_3$, 0.4% phenol) for 7 days at 4°C centrifuged to remove insoluble matter and then passed through a sterilizing Seitz and/or Millipore filter. The extracts were then di-

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alyzed against distilled water for 5 days and lyophilized. The freeze-dried extracts were reconstituted for experimental use in a buffered salt solution (140 mM NaCl, 2.7 mM KCl, 0.41 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.5 mM dextrose, 5 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid [HEPES]) containing 3.5×10^{-5} g/L of heparin and 0.1% gelatin (BSS gel). Insoluble material was removed by centrifugation.

Washed peritoneal cells were obtained from female Sprague-Dawley rats and incubated with test extracts for 20 min at 37°C in BSS-gel as previously described, using 10^5 mast cells per tube (*19*). Phopshatidyl serine (50 μg/mL) was present in the incubation mixture, to enhance histamine release (HR), except where specified. Rabbit anti-rat IgE (*20*) was also used as a positive control for the induction of HR in all experiments. For some experiments, peritoneal cells were fractionated on preformed Percoll (Pharmacia, Sweden) gradients, by a modification of the procedure described by Nemeth and Rohlich (*21*). Passive sensitization was performed by incubating peritoneal cell suspensions with rat IgE (20 μg/mL, 90 min at 37°C), followed by three washes in BSS-gel. Cells were examined for viability using trypan blue. Histamine released into the supernatant fluid was assayed by a modification of the spectrofluorimetric method of May et al. (*22*). The values of HR were calculated as percentages of the total histamine content of the cells (obtained by lysing cells with perchloric acid) after correcting for the amount of histamine released spontaneously during incubation with BSS-gel alone; this spontaneous release was less than 10%. Each HR test was performed in triplicate on each cell suspension, and the values given are the means of experiments performed on two or more different days.

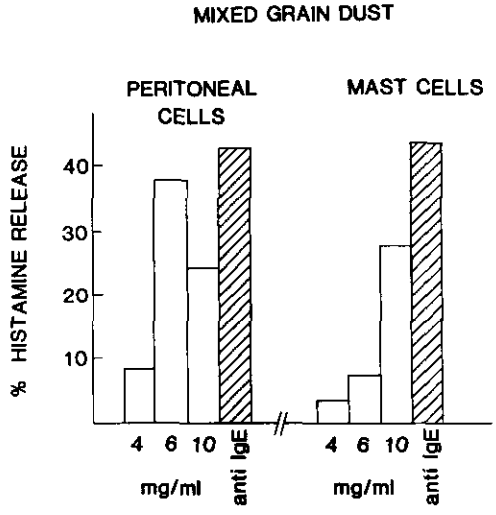

FIGURE 1. Histamine release induced by various concentrations of mixed grain dust extract and anti-IgE from peritoneal cells and purified mast cells. The values are means obtained from three experiments.

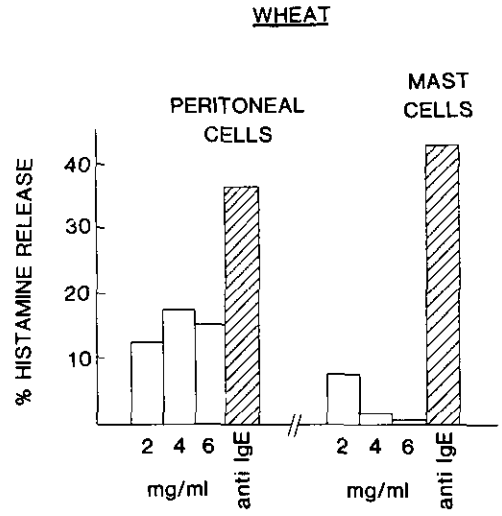

FIGURE 2. Histamine release induced by wheat extract and anti-IgE from peritoneal cells and purified mast cells. The values are means obtained from four experiments.

Results

Figure 1 shows the results of tests using the extract of mixed grain dusts collected from various elevators and the positive control of anti-rat IgE. Histamine release was induced both from unfractionated peritoneal cells and from purified mast cells by the dust extract. However, examination of the viability of the cells after the test revealed that the extract was cytotoxic. It is, therefore, possible, that the observed release was due to a nonspecific toxic effect. Dr. Ragnar Rylander, Department of Environmental Hygiene, Gothenburg, Sweden, kindly measured the endotoxin level in the extract, and it was 0.41 μg/mL, a level which may have been high enough to cause the observed effect.

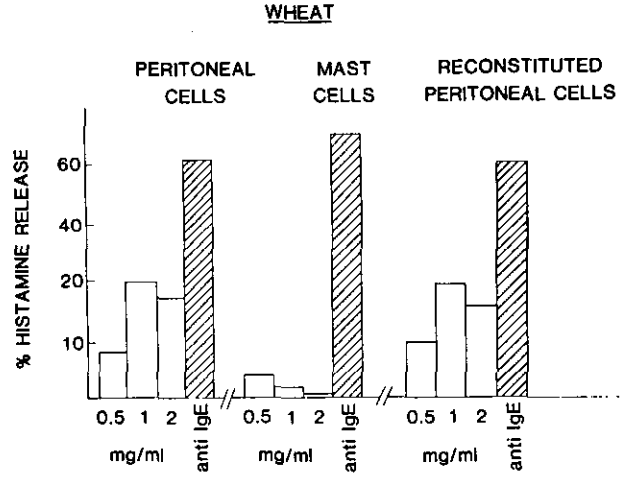

FIGURE 3. Histamine release induced by wheat extract and anti-IgE from peritoneal cells, purified mast cells, and reconstituted peritoneal cells. The values are means obtained from two experiments.

We next examined the histamine-releasing activity of the wheat extract (Fig. 2). Optimal concentrations of the wheat extract produced up to 17% histamine release from the peritoneal cells but only 7% for purified mast cells. In contrast to the findings for the mixed grain dust extract, the cells were viable, with no evidence of cytotoxic damage; Rylander found a very low endotoxin content of 0.013 μg/mL in this extract. We investigated whether the lack of histamine release from purified mast cells might reflect an adverse effect of Percoll purification on them. Figure 3 shows the results of further experiments in which the mean release from peritoneal cells was again higher than that from purified mast cells. It was clear that this low histamine release was not due to exposure of cells to Percoll, since the remixing of fractions obtained after centrifugation on the Percoll gradient (reconstituted peritoneal cells) restored the histamine release to the level obtained with unfractionated cells. It seems that for histamine to be released from mast cells by wheat extract the presence of other peritoneal cells, e.g., macrophages, lymphocytes is required.

Since IgE-mediated histamine release is potentiated by the presence of phosphatidyl serine, this phospholipid was routinely added to the buffer solutions at the time of challenge with extracts or with anti-IgE. A comparison of the histamine release obtained from cells in the presence or absence of phosphatidyl serine showed that it was required for histamine release to be induced by either wheat extract or anti-IgE (Fig. 4). In contrast, the cytotoxic mixed grain dust extract induced release in the presence or absence of phosphatidyl serine (data not shown).

In order to examine whether the wheat extract could act as an IgE-binding lectin we examined whether histamine release would be enhanced by IgE. Certain lectins of which Concanavalin A is a well-known example induce histamine release by binding to surface IgE molecules. Thus, passive sensitization of mast cells with IgE would be expected to increase histamine release. The peritoneal cells were incubated with rat IgE (20 μg/mL) for 90 min at 37°C, washed, and challenged in the usual way. Histamine release from control cells and sensitized cells are shown in Figure 5. The passive sensitization did not enhance histamine release with wheat but did with anti-IgE. It was found, in general, that the histamine release showed a bell-shaped dose–response curve to increasing concentrations of wheat extract. The reason for lower release at the higher extract concentrations is unclear, although this is a frequent occurrence in IgE-mediated release tests. We had noted that our wheat extract tended to form a precipitate in the Tyrode-gelatin buffer at higher concentrations, which may have influenced the release. We therefore examined the effect of using a Tyrode-BSA buffer. The same phenomenon of decreased histamine release at

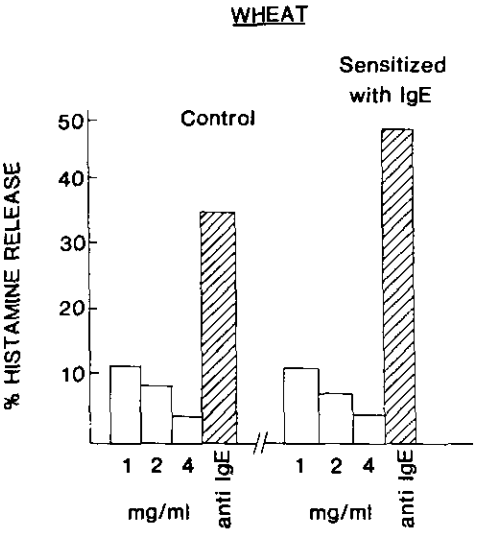

FIGURE 5. Histamine release induced by wheat extract from control cells and cells passively sensitized with rat IgE. The values are means obtained from seven experiments.

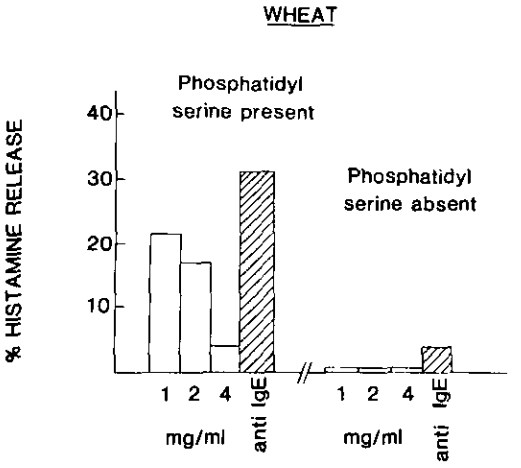

FIGURE 4. Histamine release induced by wheat extract: requirement for phosphatidyl serine. The values are means obtained from three experiments.

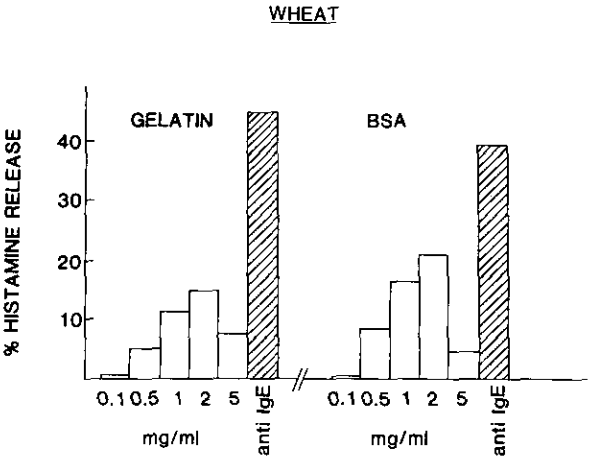

FIGURE 6. Histamine release induced by wheat extract in the presence of buffered salt solution containing gelatin or BSA. The values are from one experiment.

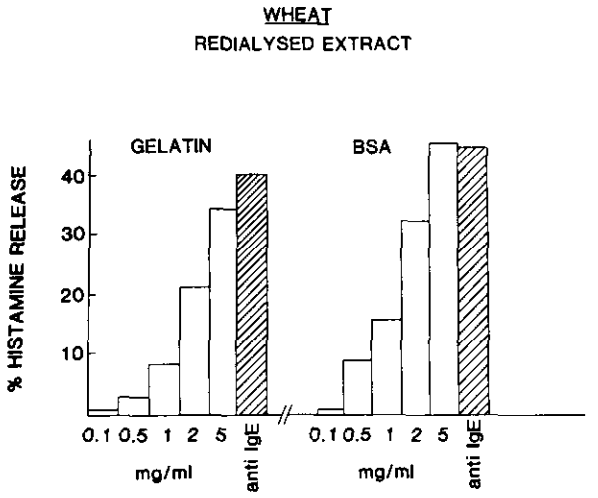

FIGURE 7. Histamine release induced by redialyzed wheat extract in the presence of buffered salt solution containing gelatin or BSA. The values are from one experiment.

higher concentration was seen, although no precipitate was found (Fig. 6). Since it was possible that the original dialysis step had not been completely effective at removing low molecular weight constituents, with potential inhibitory capacity, from the extract, we examined histamine release in the two buffers after we had dialyzed the wheat extract for a further 5 days (Fig. 7). This further dialyzed material seemed to produce a higher histamine release.

Discussion

Histamine release was induced *in vitro* by extracts of five out of ten samples of grain dust, a mixed sample of grain dust, and a sample of whole wheat grain. The concentrations of the grain dust extracts required to induce histamine release were found to be cytotoxic, and subsequent analysis indicated a relatively high endotoxin content. In contrast, the wheat extract induced noncytotoxic histamine release from unfractionated peritoneal cells and contained only low endotoxin levels. It appeared, however, that the mechanism of release by the wheat extract did not involve direct stimulation of the mast cells, since very little histamine was released from a purified mast cell preparation. Thus it appears that involvement of another cell type is required for the release process. Factors with histamine-releasing activity may be released from lymphocytes, e.g., from human peripheral lymphocytes (*23*), but it is not clear whether such factors would be produced in the short incubation time involved in conventional histamine release tests. Peritoneal cell preparations also contain cells of the macrophage/monocyte lineage which are capable of secreting numerous factors upon appropriate stimulation (*24*). Thus, it may be envisaged that the wheat extract caused the release of factors from macrophages which subsequently induced histamine release from mast cells.

The component of the wheat extract responsible for the release has not yet been identified. It seems unlikely that it would be the lectin wheat germ agglutinin (WGA) found in *Triticum vulgaris*. WGA has been shown to induce HR from rat mast cells but, in contrast to the present findings, WGA caused release from purified mast cells and did not require phosphatidyl serine for the effect (*25*). A lectin of the type exemplified by Concanavalin A (Con A), the lectin derived from the jack bean (*Canavalia ensiformis*), is able to induce HR from rat peritoneal mast cells, by virtue of its ability to bind to the IgE immunoglobulin on the cell surface (*26*). However, in this case release would also occur with purified mast cells and be potentiated by passive sensitization of mast cells with IgE, unlike the findings with wheat extract.

It should be noted that high doses of the extracts were required in order to demonstrate HR and that measurable HR occurred over a fairly limited concentration range, i.e., concentrations of 0.5 to 5 mg/mL covered the dose–response curve. In most biological systems, involving HR from mast cells, inducing agents are active over a wider range extending to far lower concentrations. It is not yet clear if this is a reflection of the low concentration of active substances in the extracts (inhibitory effects becoming apparent at higher concentrations) or if the substance itself is active at only a narrow range of doses.

Histamine release by other types of vegetable products has been demonstrated *in vitro* by several authors, using lung tissue from various species and platelets, e.g., cotton dust (*15–18*), hemp dust (*18*), western red cedar dust (*27*), but so far the active agent in these dusts has not been identified. The investigations reported here indicated that the grain dust extract tested induced histamine release from mast cells via a cytotoxic mechanism. This extract was found to contain endotoxin, which may have been responsible for some of the clinical effects caused by the dust. The extract of whole wheat grain, however, induced noncytotoxic histamine release. This supports the hypothesis that certain components of grain might contribute to the respiratory problems found in grain handlers by a histamine-releasing mechanism. However, further experiments will be required to clarify the mechanism of action and to investigate the pathogenic relevance of this effect.

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