

Non-IgE-dependent bacteria-induced histamine release from human lung and tonsillar mast cells

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Summary

A wide spectrum of formalin-killed bacteria have been tested for their ability to release histamine from human dispersed lung and tonsillar mast cells. *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Klebsiella oxytoca* and *K. pneumoniae* were the most effective histamine releasers. Further studies on tonsillar mast cells showed that *E. coli*-induced histamine release differed from IgE-dependent release with respect to its kinetics, temperature and pH profiles and its sensitivity to calcium deprivation and metabolic inhibitors. A lectin-mediated mechanism may operate, but other non-immunological mechanisms might also be involved in the release. *Escherichia coli* and anti-IgE did not synergize in inducing histamine release. The production of PGD₂ and the failure to detect lactate dehydrogenase following incubation of mast cells with *E. coli* suggests that histamine release is not due to cytotoxicity.

Introduction

Bacterial and viral infection of the respiratory tract is associated with increased non-specific airways reactivity and exacerbations of bronchial asthma [1–5]. Several mechanisms for the effects of bacteria have been suggested. The detection of IgE directed to specific bacterial antigens and the demonstration of a weal-and-flare reaction in the skin suggests that immediate and late onset bronchoconstriction following inhalation of killed *Bacillus subtilis* has an allergic basis [6]. In contrast, the early bronchoconstrictor response to *Haemophilus influenzae* has been suggested to be caused by a direct bronchial effect of its endotoxin whereas the late reaction to this bacterium may be a toxic action or a type III allergic reaction [7]. Bacterial infection has also been reported to increase α -adrenergic and decrease β -adrenergic activity [8]. Finally, it has been suggested that bacteria or bacterial products interact directly with

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inflammatory cells within the lung to induce mediator release [9,10]. Human lungs, particularly the airway mucosa and alveolar walls, are rich in mast cells, activation of which by micro-organisms may contribute to inflammation of the airways.

Recent *in-vitro* studies have shown that many species of whole formalin-killed bacteria are capable of inducing histamine release from human basophil leucocytes by non-immunological mechanisms [10,11]. Stimulation of pulmonary mast cells for secretion by a similar non-immunological mechanism represents an attractive possibility, which may contribute to exacerbations of airflow obstruction by bacterial infection, particularly as immediate bronchoconstrictor reactions to inhaled bacteria are unaffected by pre-treatment with sodium cromoglycate [12,13]. Long term bacterial colonization of the airways may also contribute to reversible airflow obstruction in chronic bronchitis, in which histological evidence for degranulation of mast cells of the bronchial mucosa has been reported [14]. Finally, an interaction between bacterial products and mediator secretory cells in the lung probably plays an important role in adult respiratory distress syndrome associated with Gram-negative septicaemia. In order to investigate these possibilities further we have examined the capacity of several strains of bacteria to release histamine from mast cells of the human respiratory tract and investigated the mechanism of action of one of these.

Materials and methods

Materials

Pronase (*Streptomyces groseus*), chymopapain (*Papaya latex*), human serum albumin (HSA, Cohn fraction V), deoxyribonuclease (DNase, bovine pancreas), 2-deoxy-D-glucose, antimycin A, N-2-hydroxyethylpiperazine-N-2-ethane-sulphonic acid (HEPES), α -methyl-D-glucoside, α -methyl-D-mannoside, N-acetyl-glucosamine, D-glucose, D-mannose, D-galactose and L-rhamnose were purchased from Sigma Chemicals, Poole, U.K. Percoll was from Pharmacia, Milton Keynes, U.K. All other chemicals including ethylenediaminetetra-acetic acid (EDTA) and trichloroacetic acid (TCA) were purchased from BDH, Poole, U.K. [^3H]-S-adenosylmethionine (15 Ci/mol) and [5,6,8,9,12,14,15, (n)- ^3H] prostaglandin (PG) D₂ (100 Ci/mmol) were purchased from Amersham International, Amersham, U.K. Rabbit anti-PGD₂ serum was purchased from Dr L. Levine, Brandeis University, Waltham, MA, U.S.A. Goat anti-human IgE was prepared and heat inactivated as previously described [15]. Whole formalin-killed bacteria were prepared from cultured bacteria, which were killed by 1.5% formalin in 0.9% NaCl, washed six times, suspended in 0.9% NaCl and then freeze dried [11,16]. The bacterial preparations used in this study were: *Enterobacter cloacae*, *Escherichia coli*, *H. influenzae* (capsule bearing type), *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Streptococcus mitior*, *Streptococcus pneumoniae*, Group A streptococci (*S. pyogenes*) and Group B streptococci (*S. agalactiae*). *Staphylococcus aureus* peptidoglycan E1369 and E2371 were prepared as previously described [16]. Minimal Essential Medium (MEM) was obtained from Flow Labs., Irvine, U.K. The composition of 10 mM HEPES buffered salt solution (HBSS) was: NaCl 137 mM, KCl 2.7 mM, NaHPO₄ 0.4 mM, glucose 5.5 mM, MgCl₂ 0.5 mM, CaCl₂ 1.8 mM and HEPES 10 mM. All bacterial products and drugs were suspended or dissolved in this buffer and adjusted to pH 7.4 unless otherwise stated.

Dispersion of human lung and tonsils

Adult patients with bronchial carcinoma and children with recurrent tonsillitis were used in this study. None of them suffered from atopy or bronchial asthma. Fresh macroscopically normal human lung, obtained at surgery for carcinoma of the lung, and tonsils from tonsillectomies, were chopped finely with scissors, washed in HBSS and resuspended in MEM (2 ml/g wet weight) containing 2 mg/ml pronase and 0.5 mg/ml chymopapain [15]. The digestion was allowed to proceed for 30 min at 37°C with occasional agitation. The freed cells were separated from the undigested tissue by sequential sieving through 600 and 80 µm nylon gauze, washed twice in HBSS and resuspended in MEM with 0.02% DNAase. The remaining tissue was digested twice more and the isolated cells from the three digests pooled. The cells were passively sensitized in 10% serum from an atopic donor for 30 min at 37°C. The cells were then washed three times and resuspended in HBSS for the release experiment. The cell dispersions from lung contained 1–5% mast cells and those from tonsils 0.03–0.06% mast cells as assessed by metachromasia using Kimura stain [17].

Histamine release experiments

In order to demonstrate bacteria-induced histamine release, $2-4 \times 10^3$ mast cells were equilibrated for 10 min at 37°C and then challenged with washed whole formalin-killed bacteria (10 mg/ml wet weight) or anti-IgE (1%). The final incubation volume was 0.2 ml. Release reactions were allowed to continue for 30 min. Spontaneous release was assessed in parallel samples incubated with buffer alone and total histamine content in aliquots, which had been frozen and thawed three times.

In order to assess the calcium and energy requirements of bacteria- and anti-IgE-induced histamine release, aliquots of $2-4 \times 10^3$ mast cells were equilibrated to 37°C for 10 min in HBSS then incubated for a further 10 min with EDTA 10 mM to chelate calcium, 2-deoxy-D-glucose 10 mM to inhibit glycolysis, anti-mycin A 1 µM to inhibit oxidative phosphorylation or HBSS alone as control. Cells were then challenged with whole formalin-killed bacteria, (10 mg/ml), or anti-IgE (1%), in a final volume of 0.2 ml as described above. In order to assess the effect of temperature on the histamine release reaction, cell aliquots containing $2-4 \times 10^3$ mast cells, were equilibrated at 4, 25, 37 or 45°C before challenge with bacteria, anti-IgE or buffer. Similarly, to assess the effect of hydrogen ion concentration on histamine release, the buffer was adjusted to pH 6.4, 6.9, 7.4, 7.9 or 8.4 with 0.1 M HCl or NaOH and the aliquots of cells washed once in buffer of new pH before final suspension and estimation of histamine release.

For time-course studies, $3-5 \times 10^6$ mast cells were suspended in 3 ml HBSS and equilibrated to 37°C for 10 min. Release was initiated by addition of 0.33 ml of whole formalin-killed *E. coli* (100 mg/ml), anti-IgE (10%) or HBSS (spontaneous control). At fixed time intervals between 1 min and 60 min, the cells were agitated and duplicate 0.05 ml samples removed into 0.2 ml of ice-cold HBSS containing 10 mM EDTA to stop the release reactions.

In order to assess the effect of desensitization of mast cells to anti-IgE on *E. coli*-induced histamine release, an aliquot containing $3-5 \times 10^6$ mast cells in 3 ml was divided into two halves. One half was washed and then resuspended in HBSS⁻ (HBSS without calcium and magnesium) before incubation for 30 min with anti-IgE at a final concentration of 1%. The cells were then separated by centrifugation and resuspended

Table 1. Histamine release from human lung and tonsillar mast cells induced by whole formalin-killed bacteria (10 mg/ml wet weight)

Bacterium	Net histamine release (% total)*	
	Lung mast cells†	Adenoidal mast cells‡
<i>E. coli</i>	14.7 ± 1.81 (12)	22.2 ± 2.6 (21)
<i>Ent. cloacae</i>	15.5 ± 2.3 (5)	21.7 ± 5.9 (4)
<i>Staph. epidermidis</i>	14.5 ± 2.1 (13)	20.2 ± 3.0 (14)
<i>P. vulgaris</i>	14.2 ± 2.4 (7)	30.8 ± 4.0 (10)
<i>K. oxytoca</i>	11.7 ± 2.1 (3)	10.1 ± 2.7 (3)
<i>K. pneumoniae</i>	11.2 ± 3.9 (4)	16.2 ± 2.3 (5)
<i>Staph. aureus</i> (WHL)	8.6 ± 1.4 (10)	9.4 (2)
<i>S. faecalis</i>	8.2 (2)	11.4 ± 2.8 (4)
<i>H. influenzae</i> (capsule bearing)	1.8 ± 1.3 (3)	3.9 ± 1.8 (5)
<i>Gp.B. streptococci</i>	3.1 ± 0.7 (3)	1.2 (2)
<i>Gp.A. streptococci</i>	-0.2 ± 0.8 (3)	7.1 (2)
<i>Strep. pneumoniae</i>	-0.3 ± 1.3 (3)	1.6 ± 1.3 (3)
<i>Strep. mitior</i>	-0.45 (2)	0.8 (2)
Anti-IgE (1% v/v)	17.9 ± 3.2 (12)	13.3 ± 1.3 (25)

* Each result is the mean ± s.e.m. of two to twenty-five experiments (indicated in parentheses) in which whole formalin-killed bacteria (10 mg/ml wet weight) were incubated with dispersed human lung or tonsillar cells for 30 min.

† Spontaneous histamine release was 7.5 ± 4.6% ($n = 12$) for lung mast cells and 4.9 ± 1.3% ($n = 25$) for tonsillar mast cells.

in complete HBSS before further challenge with *E. coli* or anti-IgE. The other half of the cell aliquot was treated similarly except that no anti-IgE was added during the initial incubation.

Mediator assays

Histamine was measured by a single isotope histamine methyltransferase assay [18] and release expressed as a percentage of total cell associated histamine corrected for spontaneous release in the absence of stimulation. Bacterial products did not interfere with this assay. Prostaglandin D₂ was measured by radioimmunoassay [19]. Bacterial products neither contained histamine nor interfered with the assays of histamine or PGD₂.

Results

Histamine release induced by whole formalin-killed bacteria from human lung and tonsillar mast cells is shown in Table 1. In each experiment, release induced by anti-IgE (1%) was used as a positive control. The results show that *E. coli*, *Ent. cloacae*, *Staph. epidermidis*, *P. vulgaris*, *K. oxytoca* and *K. pneumoniae* were the most effective histamine releasers. A similar spectrum of secretory activity of bacteria was seen in both tonsillar and lung mast cells (correlation coefficient = 0.876, $P < 0.001$). By contrast, *H. influenzae* (capsule bearing type) and streptococci were ineffective in inducing histamine release. Further studies on the mechanism of bacterial histamine release were undertaken using *E. coli*.

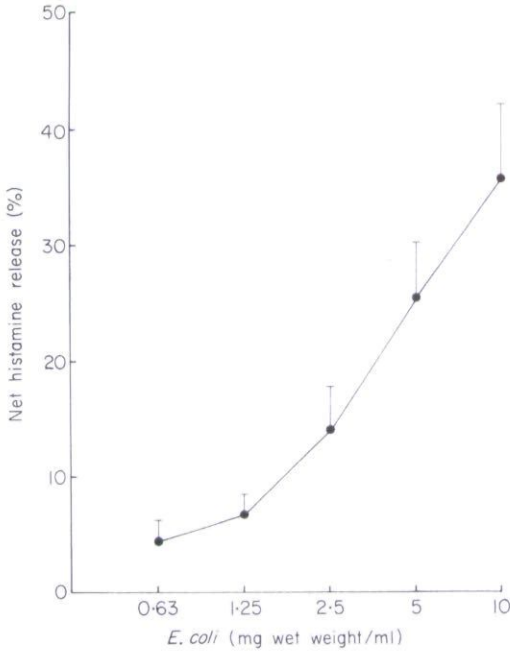


Fig. 1. Histamine release induced from human tonsillar mast cells by whole formalin-killed *E. coli*. Results are mean $\pm$ s.e.m. from six experiments in which cells were incubated with *E. coli* for 30 min. Spontaneous histamine release in these experiments was $11.2 \pm 2.7\%$.

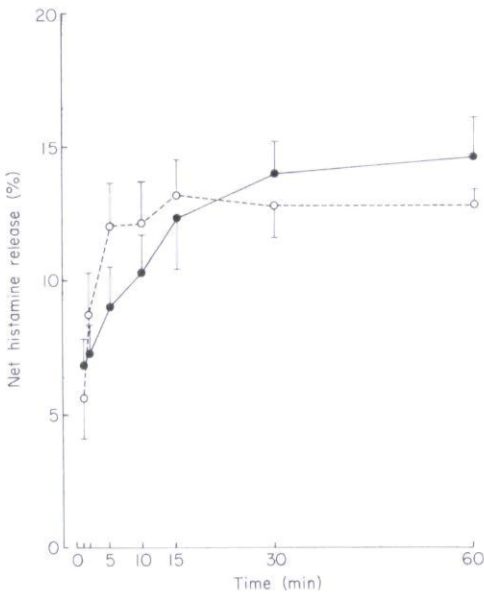


Fig. 2. Time courses for whole formalin-killed *E. coli* (●—●) and anti-IgE (○—○) induced histamine release from human tonsillar mast cells. The results show the mean net histamine release $\pm$ s.e.m. from four experiments in which cells were incubated with *E. coli* (10 mg/ml) or anti-IgE (1%).

Table 2. Calcium and energy requirements of histamine release induced from human tonsillar mast cells by whole formalin-killed *E. coli* and anti-IgE

Treatment	Net histamine release (% $\pm$ s.e.m.)	Inhibition of control (% $\pm$ s.e.m.)	Significance vs control
Whole formalin-killed <i>E. coli</i>			
Control	23.0 $\pm$ 2.1*	—	—
Ca Free + EDTA (10 mM)	16.0 $\pm$ 2.3	29.4 $\pm$ 10.6	$P < 0.05$
Glucose free + 2-deoxyglucose (10 mM)	24.3 $\pm$ 2.9	-5.4 $\pm$ 9.3	n.s.
Antimycin A (1 mM)	14.2 $\pm$ 2.6	37.7 $\pm$ 10.5	$P < 0.05$
Anti-IgE			
Control	13.6 $\pm$ 2.8	—	—
Ca Free + EDTA (10 mM)	-0.1 $\pm$ 0.9	96.4 $\pm$ 8.4	$P < 0.001$
Glucose free + 2-deoxyglucose (10 mM)	0.2 $\pm$ 0.1	98.4 $\pm$ 1.0	$P < 0.001$
Antimycin A (1 mM)	-1.9 $\pm$ 2.0	112.1 $\pm$ 17.3	$P < 0.001$

* Each result is the mean of four experiments in which cells were challenged with 10 mg/ml whole formalin-killed *E. coli* or 1% anti-IgE in complete HBSS or following 10 min pre-incubation with inhibitors as indicated. Mean spontaneous histamine release was 8.6 $\pm$ 2.2%.

Whole formalin-killed *E. coli* induced a concentration-related release of histamine from tonsillar mast cells in six experiments (Fig. 1). The relationship was linear up to the maximum concentration used (10 mg/ml) at which 35.7 $\pm$ 6.4% net histamine release occurred.

The time course of *E. coli*-induced histamine release was assessed in four experiments in which tonsillar mast cells were incubated with whole formalin-killed bacteria or anti-IgE for between 1 min and 60 min (Fig. 2). Histamine release assessed at 1 hr was similar for both secretagogues, being 14.6 $\pm$ 1.5% for *E. coli* and 12.8 $\pm$ 0.6% for anti-IgE. In contrast, with anti-IgE-induced release, which was complete within 10 min, *E. coli* induced a slow release of histamine, which plateaued at 30 min. The initial velocity of *E. coli*-induced release was 0.45 $\pm$ 0.08 net % histamine release/min compared with 1.79 $\pm$ 0.37 for anti-IgE. Analysis by paired Student's *t*-test showed these release rates to be significantly different ($P < 0.05$).

The calcium and energy requirements of histamine release induced by whole formalin-killed bacteria were compared with those induced by anti-IgE in four experiments (Table 2). Pre-incubation of cells for 10 min prior to challenge with EDTA (10 mM), in the absence of extracellular calcium or with 2-deoxyglucose (10 mM) in the absence of extracellular glucose, completely abolished the response to anti-IgE. In contrast, the secretory response to *E. coli* was only reduced by 29.4 $\pm$ 10.6% ($P < 0.05$) by calcium deprivation and unaffected by glucose deprivation. Uncoupling of oxidative phosphorylation with antimycin A (1 μ M) partially, 37.7 $\pm$ 10.5%, inhibited *E. coli*-induced histamine release whilst completely blocking that induced by anti-IgE.

The effect of variation of incubation temperature on *E. coli*- and anti-IgE-induced histamine release was assessed in three experiments (Fig. 3). With both secretagogues, little or no histamine release was observed at 4°C, and between 25°C and 37°C a

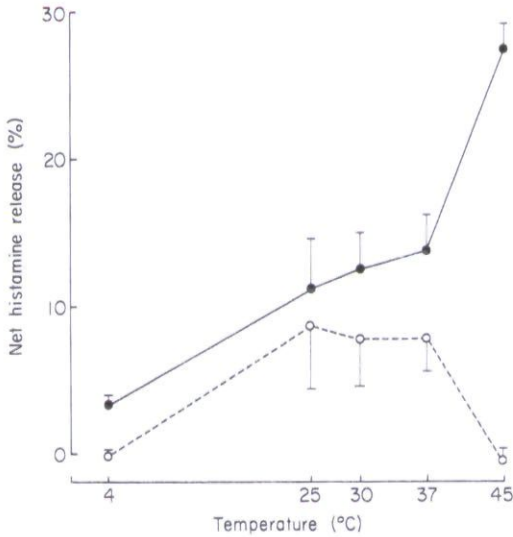


Fig. 3. Effect of incubation temperature on histamine release from human tonsillar mast cells induced by whole formalin-killed *E. coli* (10 mg/ml, ●—●) and anti-IgE (1%, ○—○). Each result is the mean $\pm$ s.e.m. net histamine release for three experiments in which spontaneous releases were $5.7 \pm 0.4\%$ at 4°C ; $4.3 \pm 1.6\%$ at 25°C ; $6.7 \pm 0.7\%$ at 30°C ; $8.7 \pm 1.7\%$ at 37°C and $11.8 \pm 2.4\%$ at 45°C .

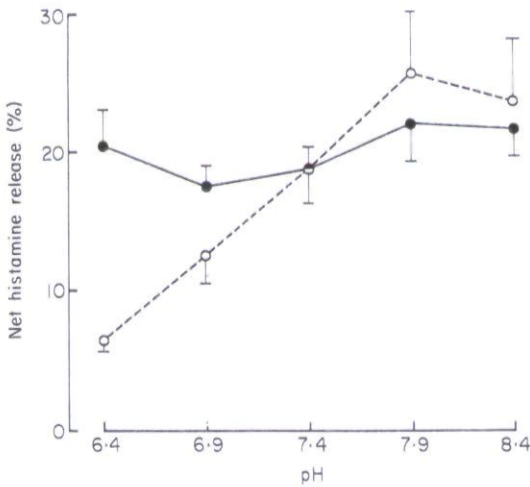


Fig. 4. The effect of pH on histamine release from human tonsillar mast cells induced by whole formalin-killed *E. coli* (10 mg/ml, ●—●) and anti-IgE (1%, ○—○). Each result is the mean $\pm$ s.e.m. for three experiments in which spontaneous releases were $18.0 \pm 3.5\%$ at pH 6.4; $17.7 \pm 1.7\%$ at pH 6.9; $20.4 \pm 2.8\%$ at pH 7.4; $20.7 \pm 2.3\%$ at pH 7.9 and $22.5 \pm 3.8\%$ at pH 8.4.

plateau of release was seen. However, increasing the temperature to 45°C caused a marked increase in histamine secretion induced by *E. coli* while suppressing that induced by anti-IgE.

The effect on pH on the histamine release reaction was assessed in three experiments (Fig. 4). There was no significant difference between histamine release induced by *E. coli* within the pH range 6.4 to 8.4. However, with anti-IgE, release was significantly less at pH 6.4 and pH 6.9 when compared to higher pH levels ($P < 0.05$).

Table 3. Prostaglandin D₂ and histamine release induced from human tonsillar mast cells by whole formalin-killed *E. coli*, anti-IgE and calcium ionophore A23187

Treatment	Net PGD ₂ release (ng/ml)		Net histamine release (%)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
WFK <i>E. coli</i> (10 mg/ml)	1640*	1340	29.2	21.3
anti-IgE (1%)	3440	1680	20.9	13.6
A23187 (1 µM)	7240	2680	24.1	14.1
Spontaneous	(1560)	(1220)	(21.5)	(20.8)

* The results show net mediator release (corrected for spontaneous release) for two experiments in which tonsillar cells were incubated with secretagogues for 30 min.

Table 4. Effect of sugars on histamine release from human tonsillar mast cells

Secretagogue	Net histamine release (%)			
	Concentration of sugar mixture (mg/ml)*			
	0	1.25	2.5	5.0
<i>E. coli</i> (5 mg/ml)	16.2 ± 4.2	13.7 ± 4.8	14.4 ± 4.8	11.3 ± 3.2
Anti-IgE (0.5%)	10.3 ± 4.5	11.2 ± 3.9	11.6 ± 4.5	14.8 ± 6.9
A23187 (0.5 µM)	14.9 ± 4.4	12.7 ± 3.1	11.8 ± 3.4	15.1 ± 1.9
Spontaneous	(7.9 ± 4.2)	(7.8 ± 3.3)	(8.2 ± 3.0)	(11.9 ± 2.1)

* The sugar mixture contained the amount stated of each of the following sugars: α-methyl-D-glucoside, α-methyl-D-mannoside, N-acetyl-D-glucosamine, D-glucose, D-mannose, D-galactose and L-rhamnose. Cells were incubated for 10 min with this mixture before induction of histamine release in three experiments.

The ability of *E. coli* to release the newly generated mast cell mediator, PGD₂, was assessed in two experiments. The results (Table 3) showed *E. coli* to be a less effective inducer of PGD₂ release than either anti-IgE or calcium ionophore A23187. For comparative purposes, PGD₂ generation (ng/ml per 1% histamine release) was 59.6 for *E. coli*, 144.1 for anti-IgE and 245.2 for A23187. In two experiments involving lung mast cells of 5 and 50% purity, *E. coli*-induced histamine release was not accompanied by release of lactic dehydrogenase.

Effect of sugars on E. coli-induced histamine release

In order to examine whether carbohydrate residues of the bacterial wall induced histamine release by a lectin-like action, two series of experiments were performed.

In the first, tonsillar mast cells were pre-incubated for 10 min with a mixture of sugars containing 1.25 to 5 mg/ml of each of α-methyl-D-glucoside, α-methyl-D-mannoside, N-acetyl-D-glucosamine, D-glucose, D-mannose, D-galactose and L-rhamnose before challenge with bacteria [20]. In three experiments (Table 4), the histamine release response to *E. coli* was reduced by $30.0 \pm 6.5\%$ at 5 mg/ml of sugar

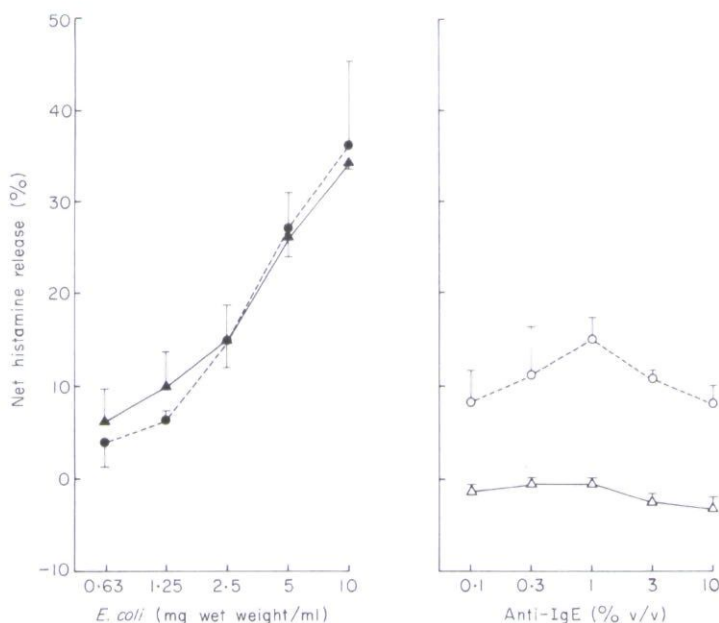


Fig. 5. The effect of desensitization to anti-IgE on histamine release from human tonsillar mast cells induced by whole formalin-killed *E. coli*. Results are mean $\pm$ s.e.m. of four experiments in which tonsillar mast cells were pre-incubated with anti-IgE (10%) in HBSS⁻ (▲ and △) or HBSS⁻ alone (● and ○) and subsequently challenged with *E. coli* (▲ and ●) or anti-IgE (△ and ○). (—) = Challenge alone; (—) = desensitized to anti-IgE.

mixture. In contrast, histamine release induced by anti-IgE and A23187 was totally unaffected by sugars.

In the second series of experiments, the ability of peptidoglycan purified from *Staph. aureus* to induce histamine release from tonsillar mast cells was examined. In four experiments, incubation of cells for 30 min with 2 mg/ml of two peptidoglycan preparations, E1369 and E2371 [16] failed to induce histamine release. In the same experiments net histamine release induced by *E. coli* (10 mg/ml) and anti-IgE (1%) was $24.8 \pm 5.4\%$ and $14.1 \pm 3.4\%$, respectively.

Interaction of *E. coli* and anti-IgE stimulation

In order to examine the possibility of whether *E. coli*-stimulated mediator secretion shared a common mechanism with IgE-dependent stimulation, interactive studies between the two secretagogues were performed. In four experiments, suspensions of tonsillar mast cells were divided into two halves, one being incubated with 10% anti-IgE for 30 min in the absence of extracellular calcium and magnesium and the other in the incomplete buffer alone. The results (Fig. 5) showed that pre-incubation with anti-IgE desensitized the cells to subsequent anti-IgE challenge in the presence of divalent cations but that release induced by *E. coli* was unaffected.

Experiments were also performed to examine whether *E. coli* could potentiate IgE-dependent histamine release and vice versa. In five experiments, anti-IgE (1%) induced $17.4 \pm 4.0\%$ histamine release and *E. coli* (2.5 mg/ml) $23.3 \pm 3.7\%$. Simultaneous addition of the two agents released $33.1 \pm 5.1\%$ histamine, significantly lower than the predicted additive release, $40.8 \pm 6.0\%$ ($P < 0.05$). Experiments were also conducted

with a synchronous addition of stimulants. In three experiments, anti-IgE stimulation was followed 15 min later by addition of *E. coli* and the release reaction allowed to proceed for a further 30 min. Histamine release was $37.7 \pm 3.7\%$. Histamine release by anti-IgE and *E. coli* separately in these experiments was $20.4 \pm 5.3\%$ and $20.1 \pm 4.9\%$, respectively, giving a predicted additive release of $40.5 \pm 4.4\%$. In three further experiments the reverse order of stimulant addition was used, i.e. *E. coli* 15 min before anti-IgE. Release here was $45.3 \pm 4.3\%$. Release induced by *E. coli* and anti-IgE individually was $26.9 \pm 5.1\%$ and $21.3 \pm 4.9\%$, respectively, giving a predicted additive release of $48.2 \pm 6.7\%$. These results provide no evidence for an interaction between the two secretagogues.

Discussion

This study demonstrates that incubation of mast cells from the human respiratory tract with killed bacteria results in their activation for mediator release. In the present studies we have used mast cells isolated from both lung and tonsils as representative sites of the lower and upper respiratory tract. In previous studies we have been unable to demonstrate any functional differences between mast cells obtained from these two sources [15,21]. The wide spectrum of bacteria that induced release, might suggest a common mechanism such as cross-linkage by bacteria IgE-antibodies bound to the mast cell surface. Such a mechanism is, however, unlikely for two reasons. Firstly, the characteristics of mediator release with bacteria were quite different from that of IgE-dependent release in respect of kinetics, temperature and pH profiles together with marked differences in the sensitivity of the two secretory stimuli to metabolic inhibitors. Secondly, it is unlikely that the two populations of tissue donors, adult patients with bronchial carcinoma and children with recurrent tonsillitis, were all similarly sensitized to the same bacteria. However, bacteria-induced histamine release caused by an IgE-dependent mechanism might occur in different patient categories since this mechanism has been demonstrated in studies of basophil histamine release in patients suffering from bronchial asthma or the syndrome of immune deficiency [10,11].

The potency order of bacteria in causing histamine release (Table 1) suggests that, in general, Gram-negative bacteria are more active than Gram-positive bacteria. It is noteworthy that two organisms that frequently colonize the respiratory tract, *H. influenzae* and group A streptococci, induced little or no histamine release. The capsule bearing form of *H. influenzae* used in these experiments did not release histamine from human basophils [16]. However, the acapsulated form, which, rather than the capsulated form, is found in asthma and chronic bronchitis, induces basophil histamine release by an IgE-dependent mechanism [10]. Furthermore, the capsulated group A streptococci also fails to release histamine from human basophils except when an IgE-dependent mechanism is demonstrable. It is possible, therefore, that the polysaccharide capsules of *H. influenzae* and group A streptococci are preventing an interaction of the bacterial cell wall with mast cells, a situation that would not occur with acapsulated or disintegrated bacteria.

As *E. coli* was amongst the most active histamine releasers in our experiments, this bacterium was used for further studies designed to elucidate its mechanism of action. In time-course studies, the concentrations of *E. coli* and anti-IgE used were those that induced a similar amount of histamine release in concentration-release studies. Under these conditions, the maximum velocity of histamine release was approximately four

times greater for anti-IgE than *E. coli*, suggesting the mechanisms of release to be different. Furthermore, whereas IgE-dependent release was optimal when pH and temperature were in the physiological range, release induced by *E. coli* was not obviously pH dependent and increased with temperature up to 45°C. The final piece of evidence that *E. coli*-induced histamine release did not involve the cross-linkage of IgE-Fc receptors was that immunological challenge of cells in the absence of extracellular calcium rendered them insensitive to subsequent anti-IgE challenge whereas the response to *E. coli* was unaffected.

The obvious question that arises from these results is whether *E. coli*-induced histamine release is a manifestation of cytotoxicity. Evidence, although not equivocal, would suggest that it is not. Firstly, *E. coli*-induced release was shown to be partially dependent on extracellular calcium and an intact oxidative phosphorylation pathway, although it did not require extracellular glucose as a source of energy. Secondly, the *de novo* synthesis of prostaglandin D₂ by mast cells would require the concerted actions of cyclooxygenase and PGD₂ synthetase enzyme, a sequence of events unlikely to occur under cytotoxic conditions. Thirdly, we found no release of lactate dehydrogenase by *E. coli* stimulation in two experiments using lung mast cells of 5% and 50% purity, respectively.

The experiments of Norn and co-workers [9,10] have suggested that bacteria can release histamine from human basophils through a lectin-mediated mechanism, i.e. that sugars on the bacterial cell wall interact with lectins on the basophil cell membrane. This mechanism has been demonstrated for mediator release induced by *S. aureus*, since the release was partially inhibited by high levels of exogenous monosaccharides and disaccharides that block lectin binding sites [11,20]. In analogy, the exogenous sugars caused partial inhibition of *E. coli*-induced histamine release without affecting the release caused by other types of stimulators such as anti-IgE and A23187 (Table 4). The inhibition of *E. coli* was smaller than by *Staph. aureus* (30% vs 45–75%), which suggests that a lectin-mediated mechanism might be of minor importance by *E. coli*-induced histamine release in adenoidal mast cells than in basophil histamine release caused by *Staph. aureus*. The finding that peptidoglycan isolated from *Staph. aureus* failed to induce release from tonsillar mast cells indicates that the suggestion of peptidoglycan sugars being involved in bacteria-induced histamine release [16] might be of no significance in adenoidal cells.

On this basis we suggest that other mechanisms than lectin-mediated reactions might also be involved in bacteria-induced mediator release. Activation of mast cells by bacterial extracts may occur by a mechanism similar to that involved in hyperosmolar-induced histamine release. Unlike hypo-osmolar lysis, hyperosmolar stimulation of basophils and mast cells is non-cytotoxic, only partially dependent on extracellular calcium and not diminished by glucose deprivation or 2-deoxy-D-glucose. In human basophils and mast cells, hypertonic stimulation markedly enhances IgE stimulation [22]. However, in our experiments with *E. coli* there was no synergistic interaction between anti-IgE and bacterial stimulation.

Finally, we cannot exclude the possibility that endotoxins in the killed bacteria preparations are responsible for mast cell activation. Such an explanation would be supported by the findings that Gram-negative bacteria were generally more active than Gram-positive organisms (Table 1). Furthermore, *E. coli* haemolysins and adhesins have been shown to participate in the release of inflammatory mediators from human polymorphonuclear granulocytes and histamine from rat mast cells [23,24]. In

contrast, endotoxins do not release histamine from human basophils but they do, however, potentiate IgE-dependent release [25].

In conclusion, we have demonstrated that whole formalin-killed bacteria induce histamine release from human lung and adenoidal mast cells. The mechanism of release was not found to be IgE-dependent but does not appear to be cytotoxic. Further experiments employing purified preparations of individual bacterial products will be necessary to elucidate the mechanism of the response.

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