

Eosinophil granule proteins inhibit substance P-induced histamine release from human skin mast cells

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We have investigated the activity of the four principal cationic proteins of the eosinophil granules, major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil-derived neurotoxin, and eosinophil cationic protein on histamine release from human skin mast cells. These four cationic proteins, over the concentration range of 10 to 200 µg/ml, did not induce significant histamine release, nor did they prime anti-IgE-induced histamine release from human skin mast cells significantly. However, a brief incubation (15 minutes) of two of the four principal eosinophil granule proteins, MBP and EPO, at concentrations of 50 to 200 µg/ml, caused a significant concentration-related inhibition of histamine release induced by 30 µmol/L substance P. The concentrations producing 50% inhibition for MBP and EPO on substance P-induced histamine release were 30 µg/ml and 100 µg/ml, respectively. This inhibitory effect appears to be a direct effect of these proteins on skin mast cells because purified (78% to 85%) skin mast cells displayed a similar response to MBP and EPO (n = 4). Also, when skin mast cells were incubated with 100 µg/ml MBP and EPO for 15 minutes and washed twice before activation by substance P, the inhibitory effect was not altered. These two proteins also inhibited histamine release induced by 10 µg/ml compound 48/80. These results suggest that MBP and EPO affect the same binding site(s) on skin mast cells as those of substance P. (J ALLERGY CLIN IMMUNOL 1994;93:900-9.)

Key words: Eosinophil granule proteins, major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), human skin mast cells, substance P, histamine release

An association between activated eosinophils and mast cells has been described in the pathology of allergic diseases such as bronchial asthma¹ and urticaria.² The granules of eosinophils contain an abundance of highly basic proteins, which are released into the extracellular fluid on activation,³⁻⁵ where they are cytotoxic to both mammalian and nonmammalian cells.⁶ These proteins are able to exert a variety of biologic actions.⁷

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Abbreviations used

ECP:	Eosinophil cationic protein
EDN:	Eosinophil-derived neurotoxin
EPO:	Eosinophil peroxidase
FCS:	Fetal calf serum
HBSS:	HEPES buffered salt solution
HEPES:	N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid
MBP:	Major basic protein
MEM:	Eagle's minimum essential medium
SPA:	Substance P antagonist, [D-Pro ⁴ , D-Trp ^{7,9,10}]SP ₄₋₁₁

Initial studies of the histamine-releasing abilities of eosinophil granule proteins focused on the ability of major basic protein (MBP) to induce histamine release from human leukocytes and rat mast cells.⁸ Native MBP at concentrations of 5×10^{-7} to 7.5×10^{-6} mol/L initiated IgE-independent, concentration- and temperature-dependent, noncytolytic histamine release from human

leukocytes and rat peritoneal mast cells, which required both calcium and metabolic energy. Eosinophil peroxidase (EPO) exhibits pronounced cytotoxic activity when combined with hydrogen peroxide and a halide, and it has been shown to be an initiator of rat mast cell degranulation.⁹ Although the amino acid sequences of eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) show a 70% homology,¹⁰ ECP but not EDN stimulated histamine release from rat mast cells.¹¹ Neither ECP nor EDN induced histamine release from human basophils.¹¹

Studies showing the marked deposition of MBP in chronic, idiopathic urticaria,¹² atopic dermatitis,¹³ onchocerciasis,¹⁴ episodic angioedema with eosinophilia,¹⁵ and the IgE-mediated late-phase reaction¹⁶ have provided evidence that eosinophil granule proteins are released and could contribute to local inflammatory processes in these diseases either directly or indirectly by activating other inflammatory cells.

Evidence suggests a direct link between neuropeptides, mast cells, and some forms of urticaria. First, substance P induces a wheal-and-flare reaction when injected intradermally.^{17, 18} Second, substance P has been shown to cause histamine release from human dispersed skin mast cells.^{19, 20} Third, the substance P antagonist [D-Pro⁴, D-Trp^{7, 9, 10}]SP₄₋₁₁ (D-Pro-Gln-Gln-D-Trp-Phe-D-Trp-D-Trp-Met-NH₂) (SPA), inhibited not only histamine release from dispersed skin mast cells²¹ but also the flare reaction induced by substance P.²² Finally, provocation challenge of patients with either cold-induced, heat-induced, or cholinergic urticaria leads to an elevation in the amounts of histamine in the venous blood drawn from the challenged but not the control arm,²³⁻²⁵ which does not occur after depletion of cutaneous neuropeptides with capsaicin.²⁶

Although it has been reported that intradermal injections of MBP, ECP, EDN, and EPO all produced dose-dependent wheal-and-flare reactions in human skin,²⁷ the effects of these proteins have not been tested directly on human cutaneous mast cells. We hypothesized that one or more of these proteins may be a human skin mast cell secretagogue or may prime mast cells for histamine release by other agents. To examine these hypotheses we incubated human dispersed skin mast cells with each of the eosinophil proteins and measured histamine in the supernatant. We also preincubated skin mast cells with eosinophil pro-

teins before the induction of histamine release with anti-IgE and substance P.

METHODS

Reagents

The following materials were purchased: collagenase (type I), hyaluronidase (type I), bovine serum albumin (fraction V), compound 48/80, dimethylsulfoxide, substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂), N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (HEPES), penicillin-streptomycin and o-phthalaldehyde (Sigma, Poole, U.K.), fetal calf serum (FCS), RPMI 1640 medium, Eagle's minimum essential medium (MEM) containing 25 mmol/L HEPES and L-glutamine (Life Technologies Ltd., Paisley, U.K.), Dulbecco's modified Eagle's medium (Northumbria Biological Ltd., Cramlington, U.K.), Percoll (Pharmacia, Uppsala, Sweden), SPA (Peninsula Laboratories, Inc., Belmont, Calif.), sheet antihuman IgE (Serotec, Oxford, U.K.). All other chemicals were purchased from BDH (Poole, U.K.).

Buffer

The HEPES buffered salt solution (HBSS) consisted of NaCl, 137 mmol/L; glucose, 5.0 mmol/L; NaH₂PO₄, 0.4 mmol/L; KCl, 2.7 mmol/L; MgCl₂, 0.5 mmol/L; CaCl₂, 1.8 mmol/L; and HEPES, 10 mmol/L. The pH was adjusted to 7.2 with 2 mol/L NaOH and 1.0% FCS added.

Purification of eosinophil granule basic proteins

The four proteins, MBP, EPO, ECP, and EDN, which constitute the main basic proteins of the specific eosinophil granule, were purified to homogeneity from eosinophils of patients with peripheral blood eosinophilia as previously described.²⁸⁻³⁰ MBP was stored in aliquots at -70° C in 0.025 mol/L sodium acetate, 0.15 mol/L NaCl (pH 4.3).⁸ EDN, ECP, and EPO were stored in aliquots at -70° C in phosphate-buffered saline.¹¹ Aliquots were thawed immediately before use, and repeated freezing and thawing steps were avoided.

Dispersal of human skin cells

Skin mast cells were dispersed from foreskins obtained at circumcision by an enzymatic procedure described previously.¹⁹ Foreskin specimens from boys aged 1 to 9 years were placed immediately in RPMI 1640 medium, stored at 4° C, and used within 24 hours of circumcision. The specimens were chopped with scissors into fragments of 0.5 to 2.0 mm³, washed twice with MEM containing 2.0% FCS (MEM/FCS), and incubated (75 minutes, 37° C) in the same buffer (1 gm tissue per 15 ml buffer) containing 1.5 mg collagenase, 0.75 mg hyaluronidase, and 25 mg bovine serum albumin per milliliter. Cells dispersed by this procedure were separated from undissociated tissue by filtration

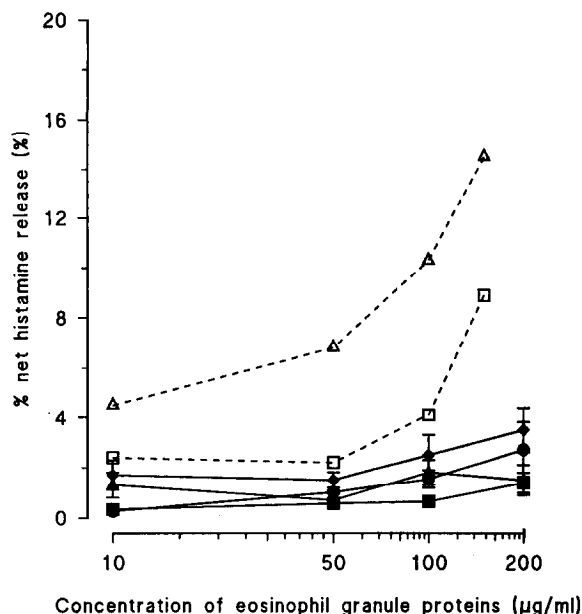


FIG. 1. Concentration-response curves in the dispersed skin mast cells with MBP (●), EPO (■), EDN (▲), and ECP (◆) in concentrations from 10 µg/ml to 200 µg/ml ($n = 4$). Skin mast cells were incubated at 37° C for 30 minutes with the indicated concentration of eosinophil granule proteins. Results are expressed as means $\pm$ SEM. To show the activity of MBP, concentration-response curves on the basophils of two individuals are shown (Δ --- Δ , $\square$ --- $\square$). Results of basophil studies are expressed as percent net of histamine release (mean of duplicate determinations from one to three experiments on each donor).

through nylon gauze (150 µm) and washed five times with MEM/FCS containing 200 U penicillin and 200 µg streptomycin per milliliter by centrifugation at 500g for 8 minutes at 20° C. Mast cell numbers were counted in a Neubauer hemocytometer after metachromatic staining with Kimura stain.³¹ With this procedure, approximately $1.0 \pm 0.1 \times 10^6$ mast cells per gram of skin in a purity 5% were obtained.

Purification of human skin mast cells

Skin mast cells were purified by density sedimentation through Percoll. Dispersed cells were resuspended in MEM/FCS and layered onto a discontinuous gradient of 60% to 80% isotonic Percoll (density 1.076 to 1.1 gm/ml) and centrifuged at 500g and 4° C for 20 minutes. The mast cells obtained from the bottom of the gradient and 70% to 80% Percoll interface were approximately 80% pure.¹⁹

Short-term culture of skin mast cells

Dispersed skin cells or purified skin mast cells were resuspended in Dulbecco's modified Eagle's medium supplemented with 2 mmol/L L-glutamine, 200 U penicillin and 200 µg streptomycin per milliliter, and 10% FCS. Cells were cultured at 2×10^5 mast cells per

milliliter in 25 cm² tissue culture flasks in humidified 95% air, 5% CO₂ at 37° C for 12 to 16 hours. Cells were washed three times in Ca²⁺, Mg²⁺-free HBSS at 20° C, counted after Kimura staining, and resuspended in an appropriate volume of HBSS before experimental use.

Mixed leukocyte preparation

Peripheral blood from informed volunteers was anticoagulated with 5 mmol/L ethylenediaminetetraacetic acid mixed with 0.25 vol of 6% wt/vol dextran, and erythrocytes were allowed to sediment for 90 minutes at room temperature.³² The plasma layer containing leukocytes and platelets was removed, and the leukocytes were pelletized by centrifugation at 100g for 10 minutes at room temperature, washed three times with Ca²⁺, Mg²⁺-free-HBSS, and resuspended in HBSS for use in the histamine release assay. Basophil numbers were assessed by counting in a Neubauer hemocytometer after metachromatic staining with Kimura stain (purity was approximately 1%).

Histamine release and assay

Histamine release was assessed in duplicate cell suspensions containing 2×10^4 basophils or mast cells. Before exposure to eosinophil granule proteins or secretagogues, cells were warmed to 37° C for 10 minutes. In release experiments 90 µl samples of cell suspension were incubated with 10 µl of eosinophil granule proteins for 30 minutes. In priming experiments on skin mast cells, 80 µl samples of cell suspension were incubated for 15 minutes with 10 µl of eosinophil granule proteins before stimulation of histamine release with 10 µl of secretagogue, to produce a final concentration of 30 µmol/L substance P, 3% anti-IgE, or 10 µg/ml compound 48/80. Release reactions were terminated 15 minutes after stimulus addition by addition of 900 µl of ice-cold Ca²⁺, Mg²⁺-free HBSS and centrifugation at 250g for 10 minutes at 4° C. The supernatant was acidified with 5% trichloroacetic acid, and histamine was measured spectrofluorimetrically.³³ Spontaneous release was estimated in duplicate tubes to which no stimuli had been added. Total histamine release was measured in parallel duplicate tubes by disrupting the cells in 5% trichloroacetic acid. Net histamine release by stimuli was expressed as a percentage of total histamine and corrected for spontaneous release.

Washing of skin mast cells before activation

In studies to investigate the point of action of MBP and EPO on substance P- and anti-IgE-induced histamine release, 80 µl samples of skin mast cell suspension were incubated with 10 µl of MBP and EPO for 15 minutes at 37° C and then washed twice with 900 µl of HBSS (at 37° C) by centrifugation at 500g for 5 minutes at 37° C. The first supernatant was saved for histamine assay. The cell pellets were resuspended in 90 µl of HBSS and immediately exposed to 10 µl

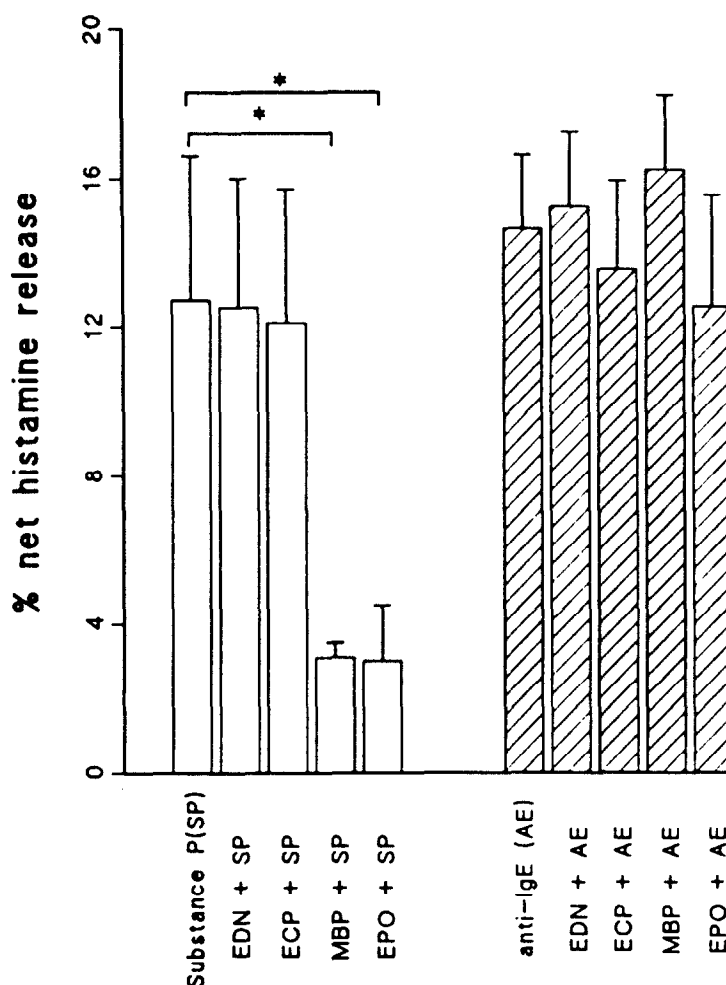


FIG. 2. Effects of eosinophil granule proteins on anti-IgE- and substance P-induced histamine release. Cells were preincubated with 100 μ g/ml MBP, EPO, EDN, or ECP for 15 minutes and then challenged by 30 μ mol/L substance P or 3% anti-IgE. Each result is the mean $\pm$ SEM of four experiments. Spontaneous release from skin mast cells was $8.0\% \pm 1.0\%$. Asterisks refer to significant difference between net control histamine release and net histamine release eosinophil granule protein treated ($*p < 0.05$).

of substance P, to produce a final concentration of 30 μ mol/L.

Substance P antagonist studies

In studies of the effects of SPA on secretagogue-induced histamine release, 80 μ l samples of cell suspension were incubated with 10 μ l of antagonist for 15 minutes at 37° C before addition of 10 μ l of secretagogue. Histamine reactions were allowed to proceed for 15 minutes at 37° C before termination as described above.

Statistical analyses

Results are expressed as means $\pm$ SEM. Differences between means were tested for significance with Student's *t* test for paired data. Differences were considered significant when the *p* value was less than 0.05.

RESULTS

Effect of eosinophil granule proteins on human skin mast cells and basophils

Incubation of dispersed skin mast cells with 10 to 200 μ g/ml of MBP, EPO, ECP, or EDN did not produce significant histamine release. Results of four experiments, each of which used skin mast cells from a different donor, are shown in Fig. 1. The highest concentration, 200 μ g/ml, of MBP, EPO, ECP, and EDN caused $2.7\% \pm 1.1\%$, $1.4\% \pm 0.4\%$, $3.5\% \pm 0.9\%$, and $1.5\% \pm 0.4\%$ net histamine release, respectively. Incubation with the vehicle used to dissolve MBP did not affect the spontaneous release. Human basophils showed an MBP-induced concentration-dependent histamine release. Because leukocyte sensi-

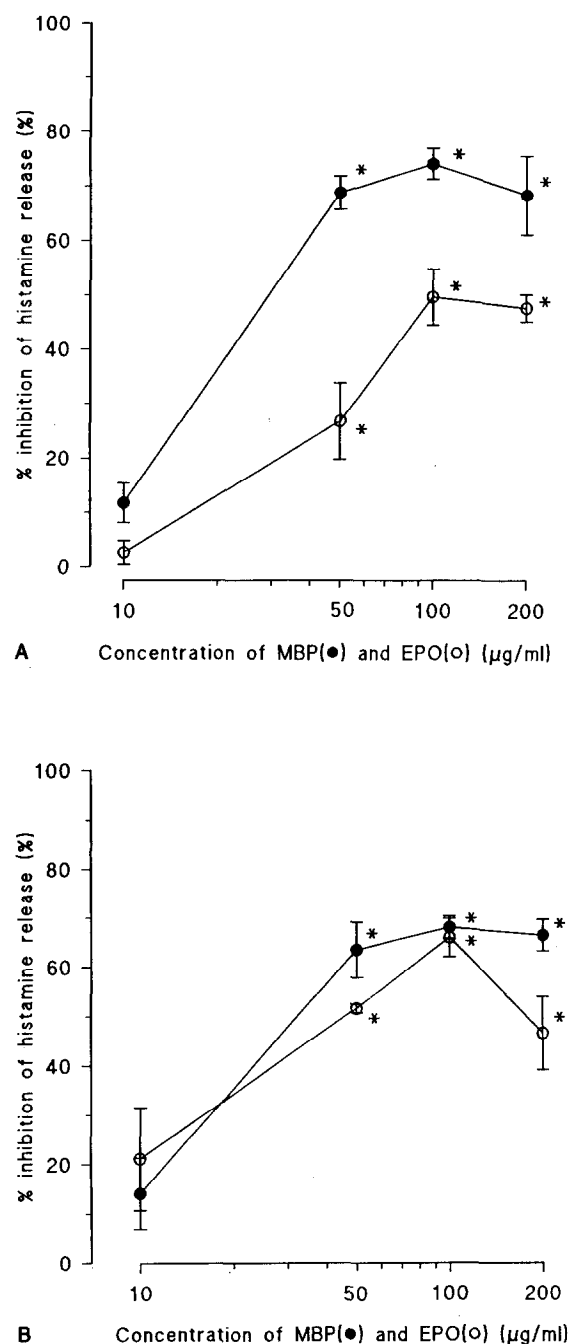


FIG. 3. Concentration-related inhibition of substance P-induced histamine release from human dispersed skin mast cells (purity was 2% to 5%) (**A**) and purified skin mast cells (purity was 78% to 85%) (**B**) by MBP (●) or EPO (○). Each result is the mean $\pm$ SEM of five (**A**) and four (**B**) experiments. Mean net control histamine releases by substance P from dispersed skin cells and purified skin mast cells were $10.8 \pm 1.3\%$ and $8.4 \pm 0.6\%$, respectively. Spontaneous releases from dispersed skin cells and purified skin mast cells were $4.0 \pm 1.5\%$ and $6.8 \pm 0.4\%$, respectively. Asterisks refer to significant difference between net control histamine release and net histamine release after MBP or EPO treatment (* $p < 0.05$).

tivity to MBP differed for individual donors, the concentration-response curves from two donors are shown in Fig. 1. Histamine release values of 14.5% and 7.9% were obtained from two individual donors' leukocytes by MBP, 150 $\mu\text{g/ml}$. In contrast, ECP, 10 to 150 $\mu\text{g/ml}$ and EDN, 10 to 150 $\mu\text{g/ml}$, did not induce a significant histamine release even from the donors whose basophils were sensitive to MBP (data not shown).

Effect of eosinophil granule proteins on anti-IgE- and substance P-induced histamine release

To investigate the possible priming effect of eosinophil granule proteins, skin mast cells were incubated with 100 $\mu\text{g/ml}$ of each granule protein for 15 minutes before activation with anti-IgE (3%) or substance P (30 $\mu\text{mol/L}$). As can be seen in Fig. 2, in four experiments with cells from four different donors, two of the four cationic proteins of eosinophil granule, MBP and EPO, inhibited substance P-induced histamine release significantly ($p < 0.05$) by $61\% \pm 10\%$ and $72\% \pm 13\%$. However, none of these proteins showed a significant effect on anti-IgE-induced histamine release. The vehicles of these proteins themselves did not affect substance P-induced histamine release.

Concentration-response study

Fig. 3, **A** shows the results of the concentration-response studies in which the skin mast cells were preincubated for 15 minutes with MBP or EPO before addition of 30 $\mu\text{mol/L}$ substance P. These two proteins, 10 to 200 $\mu\text{g/ml}$, inhibited histamine release in a concentration-dependent manner. Both MBP and EPO in concentrations of 50, 100, and 200 $\mu\text{g/ml}$ caused a significant inhibition of substance P-induced histamine release ($p < 0.05$, $n = 5$); a concentration of 100 $\mu\text{g/ml}$ showed $74\% \pm 3\%$ and $50\% \pm 5\%$ inhibition, respectively. The concentrations producing 50% inhibition for MBP and EPO on substance P-induced histamine release were approximately 30 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$, respectively.

MBP and EPO on purified skin mast cells

To establish that the inhibitory effect of MBP and EPO on substance P-induced histamine release was due to a direct effect of these proteins on human skin mast cells, four experiments were performed with preparations of enriched (78% to

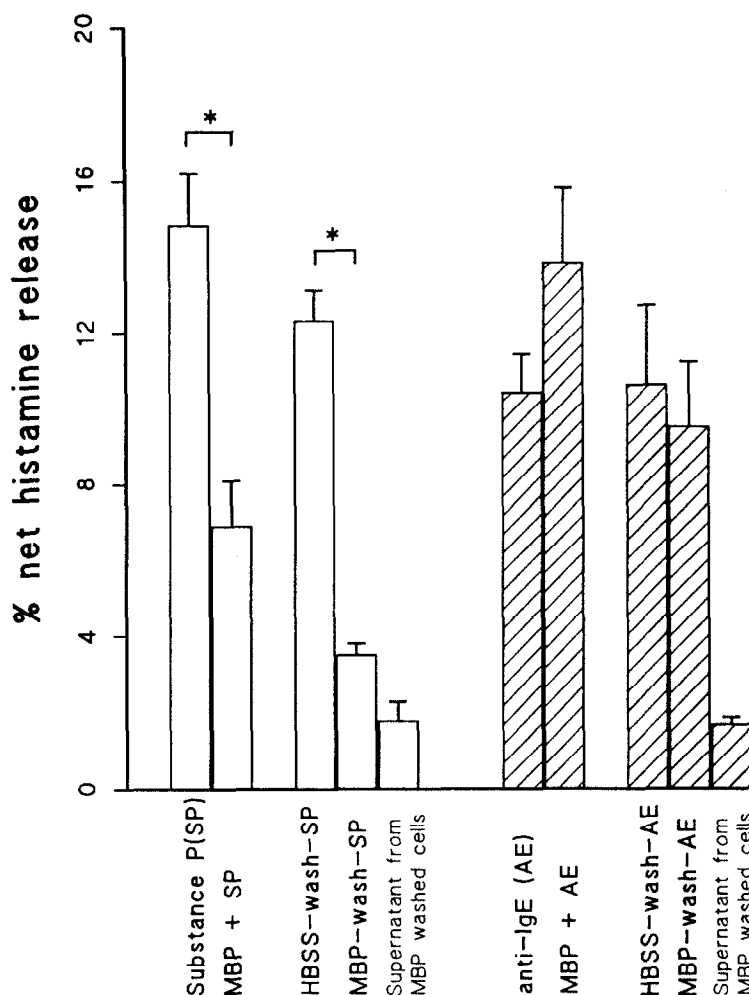


FIG. 4. Effect of washing of cells preincubated with MBP before stimulation. Cells were incubated with MBP, 100 $\mu\text{g/ml}$, for 15 minutes and washed and then challenged by substance P or anti-IgE for 15 minutes. Each result is the mean $\pm$ SEM of four experiments. Control histamine release with substance P alone was $14.8 \pm 1.4\%$, and control histamine release from washed cells with substance P alone was $12.3 \pm 0.8\%$. Spontaneous release from skin mast cells was $9.1 \pm 1.0\%$. Asterisks refer to significant difference between net control histamine release and net histamine release after MBP treatment ($*p < 0.05$).

85%) human skin mast cells. Incubation with 10 to 200 $\mu\text{g/ml}$ MBP and EPO resulted in 14% to 68% and 21% to 66% inhibition of substance P-induced histamine release respectively, similar to that seen in unpurified cells (Fig. 3, B). At the concentrations of 50, 100, and 200 $\mu\text{g/ml}$ both MBP and EPO showed a significant inhibition ($p < 0.05$).

Effect of washing of cells before activation

To determine whether MBP or EPO acts on the mast cell or whether MBP or EPO interacts with substance P, skin mast cells were incubated with

100 $\mu\text{g/ml}$ MBP or EPO for 15 minutes and washed twice before activation by substance P. In four experiments, each of which used skin mast cells from a different donor, a brief incubation with MBP before activation by substance P produced $53\% \pm 7\%$ inhibition of histamine release (Fig. 4). However, when skin mast cells were incubated with MBP and then washed before exposure to substance P, an inhibition of $73\% \pm 3\%$ was observed. The difference between these inhibitory values is likely to reflect the practical loss of histamine during washing and resuspension procedures rather than a true in-

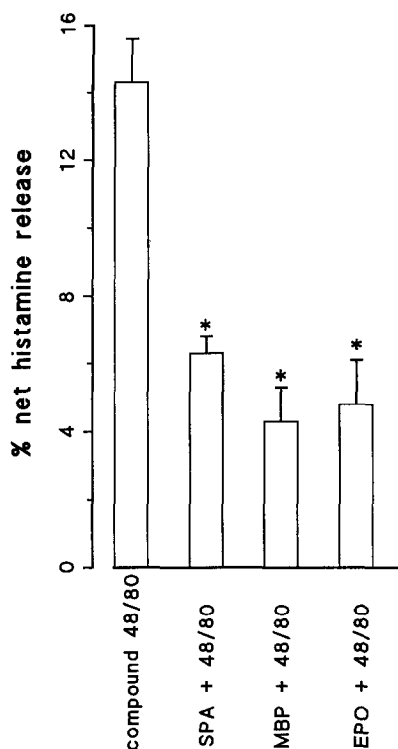


FIG. 5. Effect of MBP or EPO on compound 48/80-induced histamine release. Cells were incubated with MBP, 100 μ g/ml, or EPO, 100 μ g/ml, or SPA, 30 μ mol/L for 15 minutes and then challenged with compound 48/80, 10 μ g/ml for 15 minutes. Results are means $\pm$ SEM of three experiments. Spontaneous release from skin mast cells was $8.2\% \pm 2.1\%$. Asterisks refer to significant difference between net control histamine release and net histamine release after MBP or EPO or SPA treatment (* $p < 0.05$).

crease in the inhibitory effect of substance P. In three similar experiments, EPO, 100 μ g/ml, produced $52\% \pm 10\%$ inhibition of substance P-evoked histamine release. When skin mast cells were incubated with 100 μ g/ml EPO for 15 minutes and washed twice before activation by substance P, the inhibitory effect was not altered, being $54\% \pm 3\%$ (data not shown). In four experiments (Fig. 4), we confirmed that MBP caused a small but nonsignificant increase of anti-IgE-induced histamine release, probably as a result of the additive effects of immunologic release and the small amount of histamine release induced by MBP.

Effect of MBP and EPO on compound 48/80-induced histamine release from skin mast cells

MBP and EPO also inhibited histamine release by compound 48/80 (Fig. 5), which we have previously found to induce calcium-independent his-

tamine release from human skin mast cells.^{19, 21} In four experiments histamine release induced by 10 μ g/ml compound 48/80 was reduced by 100 μ g/ml MBP, from $14.4\% \pm 1.3\%$ to $4.3\% \pm 1.0\%$, a reduction of $71\% \pm 5\%$ ($p < 0.05$), and by 100 μ g/ml EPO, from $14.4\% \pm 1.3\%$ to $4.8\% \pm 1.3\%$, a reduction of $65\% \pm 12\%$ ($p < 0.05$). In these experiments as a positive control, histamine release by compound 48/80 was reduced from $14.4\% \pm 1.3\%$ to $6.3\% \pm 0.5\%$ in the presence of SPA, a reduction of $55\% \pm 6\%$ ($p < 0.05$).

DISCUSSION

We have shown that the four eosinophil cationic proteins, MBP, EPO, ECP, and EDN at concentrations of 10 to 200 μ g/ml did not induce significant histamine release from isolated human skin mast cells. These data reveal biochemical heterogeneity among human histamine-containing cells because previous studies on basophils have demonstrated that MBP selectively produced histamine release, whereas ECP and EDN did not.¹¹ Polymers (>13.9 kd) of L-arginine or L-lysine stimulate histamine release,^{8, 34} but other basic proteins including substance P,³⁵ compound 48/80, and polymyxin B³⁶ do not stimulate histamine release from human basophils. In contrast, human skin mast cells are unique among mast cells dispersed from human tissues in releasing histamine in response not only to polymers of L-arginine or L-lysine but also to substance P, vasoactive intestinal peptide, somatostatin, compound 48/80, and morphine.^{20, 21} The ability of neuropeptides and basic proteins to induce histamine release in human skin mast cells is closely correlated to histamine release activity from rat peritoneal mast cells. However, both MBP and ECP, but not EDN, stimulated histamine release from rat purified peritoneal mast cells,¹¹ although rat mast cell degranulation by EPO required exogenously added hydrogen peroxide and halide.⁹

The four eosinophil proteins did not prime anti-IgE-induced histamine release from human skin mast cells. We confirmed, using a washing procedure, that the small increase in anti-IgE-induced histamine release after incubation with MBP was due to an additive effect of the two stimuli. However, a brief incubation with MBP and EPO caused a significant inhibition of substance P-induced histamine release in a concentration-dependent manner. Furthermore, the results obtained with the use of purified skin mast cells and a washing procedure before activation of cells by substance P indicate that the inhibitory

effects are due to a direct influence of MBP and EPO on skin mast cells.

The inhibitory effect on substance P-induced histamine release by MBP or EPO is not simply due to the highly cationic nature of the proteins because ECP and MBP have essentially identical isoelectric point values.³⁷ These results are in agreement with those published by O'Donnell et al.⁸ who investigated the MBP-induced histamine release from peripheral leukocytes. Mild reduction and alkylation of MBP reduced its ability to induce histamine release from basophils. The distinct selectivity of MBP as an initiator of basophil histamine release and the effects of reduction and alkylation argued against net positive charge and high arginine content as the sole determinants of the biologic activity. The inhibitory effect of EPO in skin mast cells is unlikely to be due to its enzymatic activity because its substrates, hydrogen peroxide and halide, were not present in the incubates. In this regard, the inhibitory effect of substance P-induced histamine release by EPO differs from the EPO mechanism of rat peritoneal mast cell activation.⁹ Substance P has a high positive charge; its isoelectric point is approximately 9.4 because the C-terminal arginine and lysine groups are both charged at physiologic pH. Thus the eosinophil granule proteins are not likely to interact with substance P, a finding confirmed by the observation that in skin mast cells incubated with 100 µg/ml MBP or EPO for 15 minutes and washed twice before activation by substance P, the inhibitory effect was not altered. However, it is possible that their cationic charge serves to attract the four eosinophil cationic proteins to the negatively charged cell surface whereupon the apolar residues insert into and perturb the lipid milieu.

Our previous studies²¹ demonstrated that the similar characteristics of histamine release induced by substance P and compound 48/80 suggest that they share a common pathway of activation-secretion coupling. MBP and EPO also inhibited compound 48/80-induced histamine release. These results suggest that MBP, EPO, and substance P may all combine with a common cell-surface activation site of low affinity and poor specificity, the latter being an agonist, whereas the eosinophil proteins are essentially antagonists with only very weak agonist activity. A recent report by Rohrbach et al.³⁸ showed that MBP and EPO were platelet agonists with an activation mechanism distinct from that of thrombin and supports our findings that these two granule proteins share a common pathway of activation-se-

cretion coupling. Studies by Zheutlin et al.,³⁹ who attempted to further characterize the molecular basis for MBP-induced histamine release from basophils, have shown that it is pharmacologically sensitive to inhibition by a calmodulin antagonist, theophylline, pertussis toxin, and 4-bromophenacyl bromide but not by a protein kinase C inhibitor, H-7. In skin mast cells, substance P-induced histamine release is inhibited by a β-adrenoceptor agonist, protein kinase C inhibitors, cyclic adenosine monophosphate-dependent protein kinase A inhibitor H-89, pertussis toxin, and isobutylmethylxanthine but not by forskolin (El-Lati SG, Church MK. Unpublished observations). Although the nature of the activation site on human skin mast cells has not been elucidated, studies in rat mast cells have suggested that basic secretagogues, such as substance P and compound 48/80, may interact directly with membrane-associated G proteins involved with activation-secretion coupling, thus bypassing classical receptors.⁴⁰⁻⁴²

Localization of MBP in skin lesions of chronic urticaria, atopic dermatitis, and allergic late-phase reactions has implicated this protein as a participant in the inflammatory events of allergic diseases.^{12, 13, 16} Also, the cytotoxic properties of MBP and EPO provide a well established basis for proinflammatory activities of these molecules. However, mast cell activation does not appear to be one of these effects at concentrations demonstrated in the serum of atopic patients with eosinophilia⁴³ or asthma⁴⁴ and in sputum of patients with asthma.⁴⁵ At higher concentrations, which may occur focally in allergic disease, MBP and EPO may even act as local mast cell-stabilizing agents.

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Inhibition of mast cell mediator secretion induced by protoporphyrin plus long-wave ultraviolet light: A morphometric and ultrastructural analysis

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It has been shown that treatment of rat peritoneal mast cells with protoporphyrin plus long-wave ultraviolet light (UVA) irradiation can suppress mediator secretion. In this study we conducted a morphometric and ultrastructural analysis of rat peritoneal mast cells to investigate possible alterations produced by this treatment before or after stimulation with calcium ionophore. Protoporphyrin plus UVA, at doses causing inhibition of mediator release, had no effect on either cell size or viability but increased cellular sphericity. There was a 43% reduction of the cell surface area, and qualitative inspection of the cells revealed that this change was associated with a reduction in microfolds on the cell surface. After 1 minute of incubation with calcium ionophore A23187 (1 μ mol/L), both cells that were pretreated with protoporphyrin plus UVA and control cells showed dramatic changes in granule structure. Although treated cells had an unchanged tendency to have granules that closely approached the plasma membrane, there was an inhibition of granule extrusion in response to ionophore stimulation. These observations may be relevant to the inhibitory effect of protoporphyrin plus UVA on the generation and release of mast cell mediators. (J ALLERGY CLIN IMMUNOL 1994;93:909-18.)

Key words: Mast cells, protoporphyrin, long-wave ultraviolet light, plasma membrane, morphology, morphometry

We have previously shown that the treatment of rat peritoneal mast cells (RPMCs) with the endogenous photosensitizer protoporphyrin, plus long-wave ultraviolet light (UVA) irradiation, can

inhibit the release of histamine and generation of prostaglandin D₂ induced by compound 48/80, calcium ionophore A23187, or antigen. This inhibitory effect is partially dependent on extracellular calcium, is noncytotoxic, and is apparently unrelated to the generation of reactive oxygen species.¹ We and others have also shown that another photosensitizer, methoxsalen, plus UVA, inhibits skin test responses to mast cell degranulators in human subjects.²⁻⁴ However, the mechanism by which photoactive substances inhibit mast cell activation remains unclear. Protoporphyrin plus UVA (PP/UVA) can inhibit mast cell mediator release stimulated by a phorbol ester, implying that the treatment acts on steps in the stimulus-

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