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Eosinophil Granule Proteins Activate Human Heart Mast Cells[1,2]

Vincenzo Patella,* Gennaro de Crescenzo,* Isabella Marinò,* Arturo Genovese,* Monika Adt,† Gerald J. Gleich,‡ and Gianni Marone[3]*

Eosinophilia in humans is often associated with heart disease and cardiac localization of eosinophil granule proteins, and several results suggest that granule proteins mediate endomyocardial damage. Here we investigated the in vitro effects of the four principal eosinophil granule proteins (eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil-derived neurotoxin, and eosinophil peroxidase (EPO)) on the activation of effector cells of inflammation (mast cells) isolated from human heart tissue (HHMC). ECP and, to a lesser extent, MBP (0.3–3 μM), but not eosinophil-derived neurotoxin and eosinophil peroxidase stimulated the release of preformed (histamine and tryptase) and the de novo synthesis of vasoactive and proinflammatory mediators (PGD_2) from HHMC. Activation of HHMC by ECP and MBP was Ca^{2+}- and temperature-dependent and was abolished by preincubation (15 min, 37°C) with 2-deoxy-D-glucose (10 mM) and antimycin A (1 μM). There was a significant correlation between the maximal percentage of histamine release induced by ECP and anti-IgE from HHMC ($r_s = 0.73$; $p < 0.005$), by MBP and anti-IgE ($r_s = 0.79$; $p < 0.001$), and by ECP and MBP ($r_s = 0.65$; $p < 0.005$). A positive correlation was also found between histamine and tryptase secretion ($r_s = 0.71$; $p < 0.001$) and between histamine and PGD_2 release induced by ECP from HHMC ($r_s = 0.85$; $p < 0.001$). This is the first demonstration that some eosinophil cationic proteins, namely ECP and MBP, found at the site of heart damage in patients with eosinophilia, act as complete secretagogues on HHMC. This observation indicates another mechanism by which infiltrating eosinophils and their metabolic products cause inflammatory reactions and thus endomyocardial lesions in patients with eosinophilia. *The Journal of Immunology,* **1996, 157: 1219–1225.**

The association between endocardial disease and idiopathic eosinophilia was first described by Löffler in 1936 (1). Clinically detectable heart disease eventually occurs in most patients with the hypereosinophilic syndrome, and cardiac involvement is the most common cause of morbidity and mortality (2–7). Activated eosinophils (8) and eosinophil granule proteins (8, 9) have been found in endomyocardial biopsies and necroscopy samples from patients with eosinophilic endomyocardial disease. Eosinophil granule cationic proteins have been implicated in the progression of endomyocardial lesions; indeed, deposits of major basic protein (MBP)[4] and eosinophil cationic protein (ECP) are found in areas of cardiac injury (9, 10). There is compelling evidence that eosinophils and their inflammatory mediators mediate endomyocardial damage in various animal (11–14) and human experimental models (15–17), albeit through different biochemical and immunologic mechanisms (11–17).

Four principal eosinophil granule proteins have been characterized: MBP, ECP, eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase (EPO) (18). These proteins are cationic, with isoelectric point values of ≈9 for EDN and ≈11 for MBP, ECP, and EPO (19). The cationic nature of these molecules would allow them to interact with the anionic barrier found on endothelial cells and basement membranes and to influence microvascular permeability (20, 21). Alternatively, cationic molecules may indirectly affect microvascular permeability by stimulating perivascular mast cells or other vascular mural cells (22, 23) to release vasoactive and proinflammatory mediators (24, 25).

Initial studies of the histamine-releasing ability of eosinophil granule proteins focused on the ability of MBP to induce histamine release from human basophils and rat mast cells (26). Furthermore, ECP, but not EDN, stimulated histamine release from rat mast cells (27). In contrast, the four principal cationic proteins (MBP, ECP, EPO, and EDN) did not induce significant histamine release, nor did they enhance anti-IgE-induced histamine secretion from human skin mast cells (28).

Recently, we described an enzymatic procedure whereby we partially purified and immunologically characterized the mast cells isolated from human heart tissue (29). Cardiac mast cells obtained with this procedure are viable and they release preformed (histamine and tryptase) and newly generated (prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4)) mediators after stimulation with immunologic stimuli (29, 30). Although some features of HHMC are

*Division of Clinical Immunology, University of Naples Federico II, School of Medicine, Naples, Italy; †Deutsches Herzzentrum Berlin, Berlin, Germany; and ‡Departments of Immunology and Internal Medicine, Mayo Clinic and Mayo Foundation, Rochester, MN 55905.

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[3] Address correspondence and reprint requests to Dr. Gianni Marone, Division of Clinical Immunology, University of Naples Federico II, Via S. Pansini 5, 80131 Napoli, Italy.

[4] Abbreviations used in this paper: MBP, major basic protein; anti-IgE, rabbit IgG anti-Fcε fragment of human IgE; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase; HHMC, human heart mast cells; LTC_4, sulfidopeptide leukotrienes C_4; P, a mixture of 25 mM PIPES, 110 mM NaCl, and 5 mM KCl, pH 7.37; P2CG, PIPES buffer containing 2.0 mM $CaCl_2$ and 1 g/L D-glucose; PGD_2, prostaglandin D_2; PGMD, PIPES buffer containing 0.25 g/L $MgCl_2 \cdot 6H_2O$, 10 mg/l DNase, and 1 g/L gelatine; LDH, lactate dehydrogenase; rhC5a, human recombinant C5a.

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similar to those of other human mast cells, the pattern of responsiveness to different secretagogues and the amounts of mediators synthesized are unique (25, 29, 30). We therefore investigated the in vitro ability of the four eosinophil granule proteins to induce the release of preformed (histamine and tryptase) and de novo synthesized (PGD_2) vasoactive and inflammatory mediators from mast cells isolated from human heart.

Materials and Methods

Reagents

The following were purchased: 60% $HClO_4$ (Baker Chemical Co., Deventer, The Netherlands); BSA, PIPES, hyaluronidase, collagenase type II, chymopapain, elastase type I, human recombinant C5a (rhC5a), synthetic PGD_2, (Sigma Chemical Co., St. Louis, MO); HBSS and FCS (Life Technologies, Grand Island, NY); DNase I and pronase (Calbiochem, La Jolla, CA); RPMI 1640 with 25 mM HEPES buffer, Eagle's MEM (Flow Laboratories, Irvine, Scotland); Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden); [^{3}H]LTC_4 (39.3 Ci/mmol) and [^{3}H]PGD_2 (210 Ci/mmol) (New England Nuclear, Boston, MA). Rabbit anti-human Fcϵ Ab was a generous gift from Drs. Teruko and Kimishige Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA). The rabbit anti-PGD_2 Ab was kindly donated by Dr. L. M. Lichtenstein (The Johns Hopkins University, Baltimore, MD). The tryptase RIA kit (Pharmacia Tryptase RIACT 50, Pharmacia Diagnostics AB, Uppsala, Sweden) was kindly donated by Kabi Pharmacia SpA (Milan, Italy).

Buffers

The PIPES buffer used in these experiments was a mixture of 25 mM PIPES, 110 mM NaCl, 5 mM KCl, pH 7.37, referred to as "P". P2CG contains, in addition to P, 2 mM $CaCl_2$ and 1 g/L dextrose (31); pH was titrated to 7.4 with sodium bicarbonate. PGMD is 0.25 g/L $MgCl_2 \cdot 6H_2O$, 10 mg/L DNase, and 1 g/L gelatine in addition to P, pH 7.37.

Isolation and partial purification of human heart mast cells

The heart tissue used in this study was obtained from patients (27 to 66 yr) undergoing heart transplantation at the Deutsches Herzzentrum, Berlin, mostly for cardiomyopathy and from donors without cardiovascular disease who died in car accidents. The explanted heart was immediately immersed in cold (4°C) cardioplegic solution, shipped to Naples, Italy by air (4°C), and processed within 5 to 18 h of removal. The heart tissue (100 to 600 g) was dissected to separate the left and right ventricles and the septum. Fat tissue, large vessels, and pericardium were removed. The tissue was finely minced (2- to 5-mm fragments), suspended in P buffer (10 ml/g of wet tissue), and washed three times by centrifugation (the first time, 150 × *g*, 4°C, 8 min and twice at 150 × *g*, 22°C, 8 min). After each centrifugation, the heart fragments were filtered through a 150-μm pore Nytex cloth (Tetko, Elmsford, NY). Fragments were incubated (15 min, 37°C) under constant stirring in P buffer containing 10 mg collagenase/g of wet tissue. At the end of the first incubation, the cell suspension was filtered through a 150-μm pore Nytex cloth. The residual tissue was weighed, and three additional cycles of enzymatic digestion were performed. A new preparation of collagenase was used each time. After the last enzymatic digestion, the cell suspension was centrifuged (150 × *g*, 22°C, 8 min) and filtered first through a 150-μm pore Nytex cloth and then through a 60-μm pore Nytex cloth to remove large particles and large cells (mostly myocytes). Finally, cells were washed twice in PGMD (25 mM P, 110 mM NaCl, 5 mM KCl, 1 mM Mg, 1 g/L gelatin, 20 mg/L DNase, pH 7.37) by centrifugation (150 × *g*, 22°C, 8 min). At this stage of the procedure Alcian blue-positive cells (mast cells) represented <0.1% of total cells (29). Cell pellets were resuspended in 250 ml of P buffer containing 2% BSA and centrifuged (25 × *g*, 22°C, 2 min) to remove sedimented myocytes. Myocytes (>100 μm long) were pelleted and discarded; supernatants containing endothelial cells, fibroblasts, and mast cells were then collected and centrifuged (150 × *g*, 22°C, 8 min). At this stage of purification, Alcian blue-positive cells represented <1% of the total cells.

HHMC were partially purified by flotation through a discontinuous Percoll gradient. Isotonic Percoll was prepared by mixing 9 parts Percoll and 1 part 10× P solution. This mixture was then diluted with isotonic P to give Percoll concentrations of 40, 50, 60, 70, 80, and 90%. The cell suspension was overlaid on the Percoll gradient in 50-ml polypropylene tubes, and the mixture was centrifuged (350 × *g*, 22°C, 20 min). The cells found at the interface between the 60 to 70% and 70 to 80% fractions were removed and washed twice with P. Mast cells in these populations ranged from 0.1 to 18%, with an average of 12.3 ± 0.9%. The enzymatic dispersion of tissue yields ≈4.5 × 10^4 mast cells/g of heart tissue. Short-term (≈16 h) cultures of HHMC were prepared resuspending (2 to 5 × 10^6 cells/ml) in a solution of RPMI 1640 containing 25 mM HEPES, 1% penicillin-streptomycin solution, 2 mM L-glutamine, and 10% FCS at 37°C in humidified 95% air/5% CO_2. The experiments presented in this paper were performed with Percoll-enriched preparations unless indicated otherwise.

Purification of eosinophil granule proteins

MBP, ECP, EPO, and EDN were purified from eosinophils obtained from patients with marked eosinophilia as described (32, 33). In brief, eosinophils were lysed with sucrose and heparin, and granules were isolated by centrifugation. Granules were lysed by exposure to 0.01 M HCl, pH 2, and by sonication. Granule extracts were applied to a 1.2 × 47-cm Sephadex G-50 column that had been equilibrated with 0.025 M sodium acetate and 0.15 M NaCl, pH 4.3. The void volume peak fractions from the Sephadex G-50 column were pooled and further purified by ion exchange chromatography using carboxymethyl-Sepharose to obtain EPO (34). The second peak fractions from Sephadex G-50 column containing ECP and EDN were dialyzed against PBS, pH 7.4, applied to a heparin-Sepharose CL-6B column, and eluted with a linear gradient of 0.075 to 1.5 M NaCl in 0.005 M PBS, pH 7.0. Fractions containing EDN eluted at 0.15 to 0.2 M NaCl and those containing ECP eluted at 0.35 to 0.65 M NaCl (35). EDN and ECP were dialyzed against H_2O, lyophilized, and reconstituted in PBS. The third peak fractions from Sephadex G-50 column were pooled as MBP (36). The purified proteins were stored at −70°C, and samples were thawed immediately before use. Each of the proteins was pure as judged by its banding pattern on SDS-PAGE after staining with Coomassie brilliant blue R. Protein concentrations were determined using the appropriate E_{277} values (34–37). Preliminary experiments demonstrated that the buffer used, either acetate buffer (0.0025 M sodium acetate and 0.15 M NaCl, pH 4.3) or PBS had no detectable effect on HHMC viability or degranulation at the dilutions used in these studies.

Histamine release assay

Cells (≈3 × 10^4 mast cells/tube) were resuspended in P2CG, and 0.3 ml of the cell suspension was placed in 12- × 75-mm polyethylene tubes and warmed to 37°C; 0.2 ml of each prewarmed releasing stimulus was added, and incubation was continued at 37°C for 30 min (38). At the end of this step, the reaction was stopped by centrifugation (1000 × *g*, 22°C, 2 min), and the cellfree supernatants were stored at −70°C for subsequent assay of histamine, tryptase, and PGD_2 content. The cellfree supernatants were assayed for histamine with an automated fluorometric technique (39). To calculate histamine release as a percentage of total cellular histamine, the "spontaneous" release from mast cells (2 to 12% of the total cellular histamine) was subtracted from both numerator and denominator. The total histamine content in mast cells was obtained by cell lysis with 8% $HClO_4$ (40). All values are based on the means of duplicate or triplicate determinations. Replicates differed from each other by less than 10% in histamine content.

RIA of tryptase and PGD_2

Total tryptase content was assessed by lysis induced by incubating cells with 100 μl of Triton X-100 (0.1%). Tryptase was analyzed by a solid-phase RIA (Pharmacia Tryptase RIACT 50) (41). PGD_2 was analyzed on 100-μl fractions taken from the supernatant fluids. The samples were stored at −70°C. PGD_2 was analyzed by RIA (42) within 24 h of the experiment to minimize degradation of the compound (43). The anti-PGD_2 Ab is highly selective, with less than 1% cross-reactivity to other eicosanoids (42, 43).

LDH assay

To test whether the effects of ECP, MBP, EDN, and EPO on HHMC are cytotoxic, the levels of LDH in the supernatants of HHMC were determined. The cellfree supernatants were collected, and the concentrations of LDH were measured as previously described (44).

Statistical analysis

The results are mean ± SEM. Statistical analysis was performed by two-way nonparametric analysis of variance (Friedman χ_r^2 test). The extended Tukey test was used for multiple comparisons. The level of statistical significance used was $p < 0.05$ (45).

Results

Effect of eosinophil granule proteins on histamine release from HHMC

We evaluated the effects of increasing concentrations of ECP, MBP, EDN, and EPO on histamine release from HHMC. ECP and,

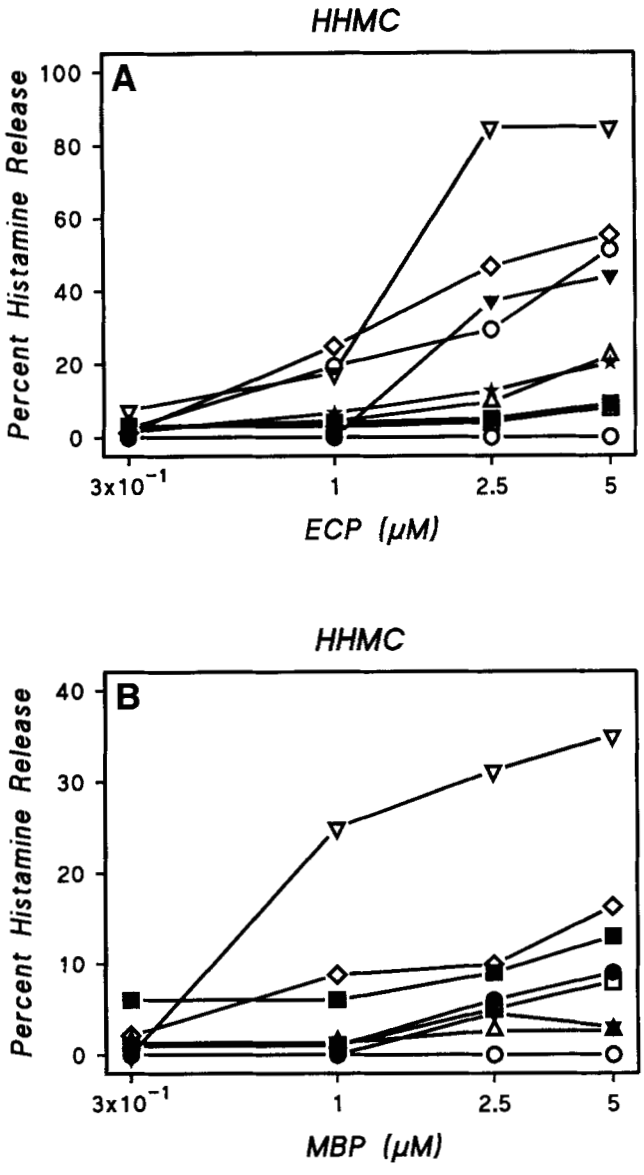

FIGURE 1. *A*, Effect of increasing concentrations of ECP on histamine secretion from HHMC obtained from nine different donors. Each symbol represents the results obtained with a different donor. Each point represents the mean of duplicate determinations. *B*, Effect of increasing concentrations of MBP on histamine secretion from HHMC obtained from eight different donors. Each symbol represents the results obtained with a different donor. Each point represents the mean of duplicate determinations. The symbols used in *A* and *B* do not refer to the results obtained with cells from the same preparations.

to a lesser extent, MBP caused a concentration-dependent histamine release from HHMC in the concentration range of 0.3 and 5 μM (Fig. 1). EDN and EPO did not induce significant histamine release (data not shown). In 20% of the experiments, HHMC did not release histamine after ECP and MBP challenge. In all other experiments, the percentage of release varied from 2 to 80%. Figure 2 compares the maximum percentage of histamine release from HHMC caused by ECP, MBP, EDN, EPO, and two other immunologic stimuli (anti-IgE and rhC5a). The maximum release caused by ECP was comparable with that caused by anti-IgE, whereas MBP caused approximately the same percentage of release as rhC5a; the release caused by EDN and EPO was less than 5%. Similar results were obtained with unpurified (<1% of the

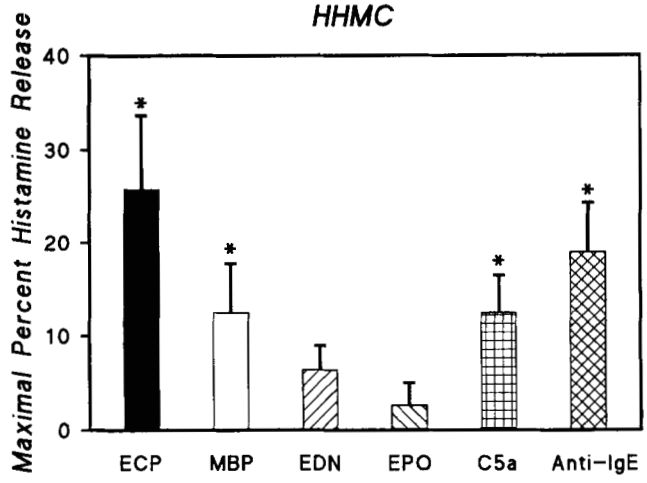

FIGURE 2. Effects of ECP, MBP, EDN, EPO, rhC5a and anti-IgE on the maximum percent histamine secretion from HHMC obtained from nine different donors. HHMC were challenged in vitro with increasing concentrations of ECP, MBP, EDN, and EPO (from 3×10^{-1} to 5 μM), rhC5a (from 10^{-7} to 10^{-6} M), and anti-IgE (from 1 to 5 μg/ml). Each bar represents the mean ± SEM. *$p < 0.01$ when compared with spontaneous release.

total cells) preparations of HHMC and from heart mast cells obtained from subjects not affected by cardiovascular disease who died in car accidents (data not shown).

Characteristics of ECP- and MBP-induced mediator release from HHMC

We next analyzed the characteristics of release caused by ECP and MBP from HHMC. The temperatures of the release reaction was similar to those observed for anti-IgE (29), in that little or no release occurred at 22°C or at 4°C, and maximal release occurred at 37°C (data not shown). In contrast, the Ca^{2+}-dependence of the release reaction was different from that observed for anti-IgE. The release of histamine induced by ECP and MBP from HHMC was highly dependent on extracellular Ca^{2+}. The absence of added extracellular Ca^{2+} abolished the response to ECP and as the extracellular Ca^{2+} concentrations increased from 0.1 to 5 mM there was a concentration-dependent increase in the release of histamine without a plateau being reached. In contrast, release caused by anti-IgE was optimal at 2 mM extracellular Ca^{2+} (Fig. 3). Similar results were obtained with MBP (data not shown). All subsequent experiments were performed at a physiologic concentration of Ca^{2+} (2 mM) and at 37°C.

Also the kinetics of ECP- and MBP-induced histamine release from HHMC differed significantly from that of anti-IgE. The kinetics of ECP-induced histamine release from HHMC were rapid ($t_{1/2}$ = 23 ± 2 s) reaching a maximum 60 s after the addition of stimulus, whereas anti-IgE-induced release was slower ($t_{1/2}$ = 170 ± 10 s; $p < 0.001$), being complete after 300 s (Fig. 4). Despite these biochemical differences, there was a linear correlation between the maximum percentage of release caused by ECP and anti-IgE ($r_S = 0.73$; $p < 0.001$), MBP and anti-IgE ($r_S = 0.79$; $p < 0.001$), ECP and MBP ($r_S = 0.65$; $p < 0.005$) (Fig. 5).

Tryptase release from ECP-activated HHMC

Tryptase is a neutral protease that is a selective marker for mast cells. Large quantities reside in the secretory granules of all mature human mast cells (≈11 pg/lung-derived mast cell and 35 pg/skin-derived mast cell) (46, 47). We recently demonstrated that HHMC

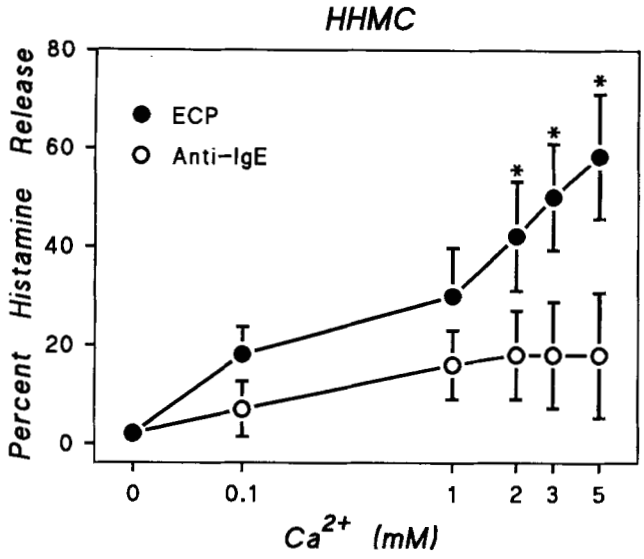

FIGURE 3. Effects of increasing concentrations of extracellular Ca^{2+} concentrations on the release of histamine caused by ECP and anti-IgE from HHMC. Each bar represents the mean ± SEM of four experiments. *$p < 0.01$ when compared with the corresponding value obtained with anti-IgE.

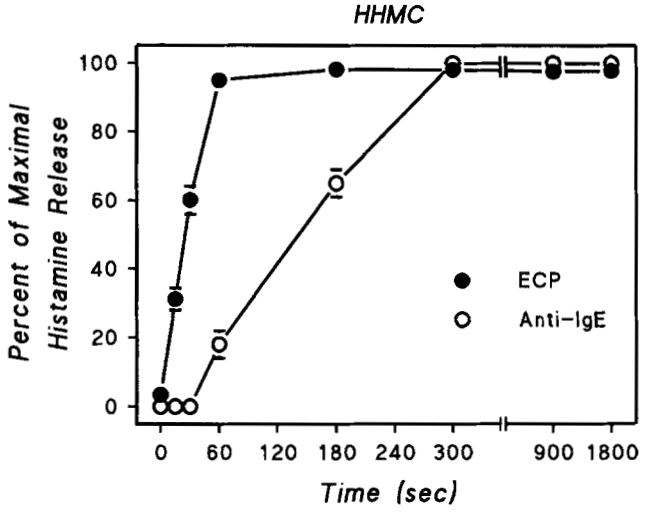

FIGURE 4. Kinetics of histamine release induced by ECP (2.5 μM) and anti-IgE (1 μg/ml) from HHMC. Each point represents the mean ± SEM of three experiments. The maximum percentage of histamine release was 45.6 ± 7.8% (ECP) and 24.2 ± 9.1% (anti-IgE). Error bars are not shown when graphically too small.

contain ≈24.2 ± 4.3 $\mu g/10^6$ cells of tryptase (29). Activation of HHMC with ECP caused the release of tryptase as well as of histamine (Table I). There was a positive correlation between the percentage of histamine and tryptase release induced by ECP (r_S = 0.71; $p < 0.001$). MBP also induced the release of tryptase from HHMC (5 to 15 $\mu g/10^7$ cells; see also Fig. 8). These data demonstrate that tryptase contained in mast cells is released in parallel with histamine, suggesting that mast cells are the main source of tryptase and histamine found in the supernatants of ECP- and MBP-activated HHMC.

De novo synthesis of PGD_2 from HHMC activated by eosinophil granule proteins

In 10 experiments, we evaluated the production of the newly formed lipid mediator (PGD_2) in response to increasing concentrations of ECP, MBP, EDN, and EPO. Figure 6 shows that ECP and, to a lesser extent, MBP caused the de novo synthesis of PGD_2. EDN and EPO did not stimulate a significant production of PGD_2 (data not shown). Activation of HHMC by ECP and MBP led to the production of 0.4 to 76.8 ng $PGD_2/10^6$ cells (29.2 ± 9.6 and 19.1 ± 5.9, respectively). There was a significant correlation between the maximum percentage of histamine secretion and de novo synthesis of PGD_2 caused by ECP from HHMC (r_S = 0.85; $p < 0.001$; n = 19) (Fig. 7). This correlation indicates that histamine contained in HHMC is released in parallel with the de novo synthesis of PGD_2, the main cyclooxygenase metabolite of HHMC, suggesting that mast cells are the main source of PGD_2 found in the supernatants of ECP-activated HHMC. Similar results were obtained with MBP (data not shown).

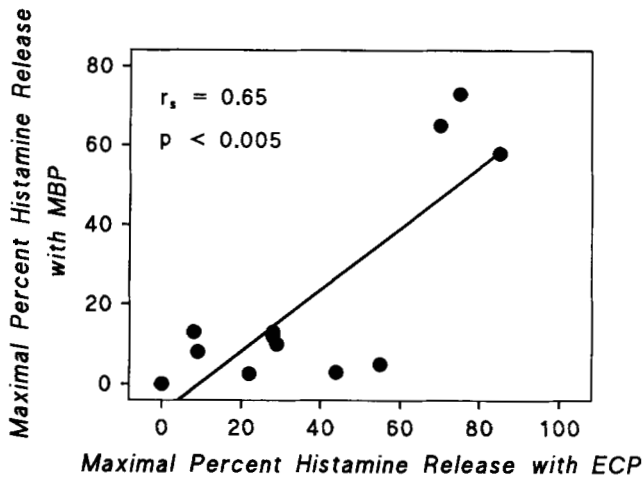

FIGURE 5. Correlations between the maximum percent histamine release from HHMC caused by ECP and MBP. Each point represents the mean of duplicate determinations from separate experiments.

Effect of metabolic inhibitors on the release of preformed and de novo synthesized mediators caused by ECP and MBP from HHMC

High concentrations of MBP and ECP are cytotoxic for mammalian cells (48–51). We therefore evaluated by different criteria the cytotoxicity of MBP and ECP on HHMC. In a first series of three experiments, cell suspensions containing enriched preparations of HHMC were tested for viability with the trypan blue exclusion method (52) after incubation (30 min, 37°C) with ECP and MBP (1–5 μM). Cell viability in the presence of these compounds was ≥95%. In a second group of three experiments, we evaluated lactate dehydrogenase (LDH) in the supernatants of HHMC incubated (30 min, 37°C) with buffer or ECP and MBP (1–5 μM). Exposure of cells to ECP or MBP did not increase the LDH content in the supernatants of HHMC. In the third group of experiments we evaluated the effect of metabolic inhibitors (2-deoxy-D-glucose and antimycin A) on the release of preformed and de novo synthesized mediators from HHMC caused by ECP, MBP, and anti-IgE. The release of tryptase and the de novo synthesis of PGD by both ECP and MBP was highly dependent on an intact glycolytic pathway. Figure 8 shows a typical experiment out of three performed with similar results in which the requirement for glycolysis was evident, as shown by a marked inhibition of ECP- and MBP-mediated release of tryptase and of the de novo synthesis of PGD_2 after cell preincubation (15 min, 37°C) with 2-deoxy-D-glucose (10 mM) and antimycin A (1 μM).

Table I. *Effects of increasing concentrations of ECP on histamine and tryptase release from human heart mast cells*[a]

ECP (μM)	Expt. 1		Expt. 2		Expt. 3		Expt. 4	
	% HR[b]	Tryptase (μg/10^7 cells)	% HR	Tryptase (μg/10^7 cells)	% HR	Tryptase (μg/10^7 cells)	% HR	Tryptase (μg/10^7 cells)
0.3	1.6	0	2.7	0	3.0	4.1	0	0.3
0.5	6.6	3.0	4.4	3.6	3.0	4.6	0	12.2
2.5	12.6	3.5	9.5	8.0	4.0	8.2	37.1	42.1
5	20.1	2.7	22.0	16.2	8.0	8.2	43.9	37.3

[a] Each result is the mean of duplicate determinations in which cells were incubated with the indicated concentrations of ECP for 30 min at 37°C.
[b] HR, histamine release.

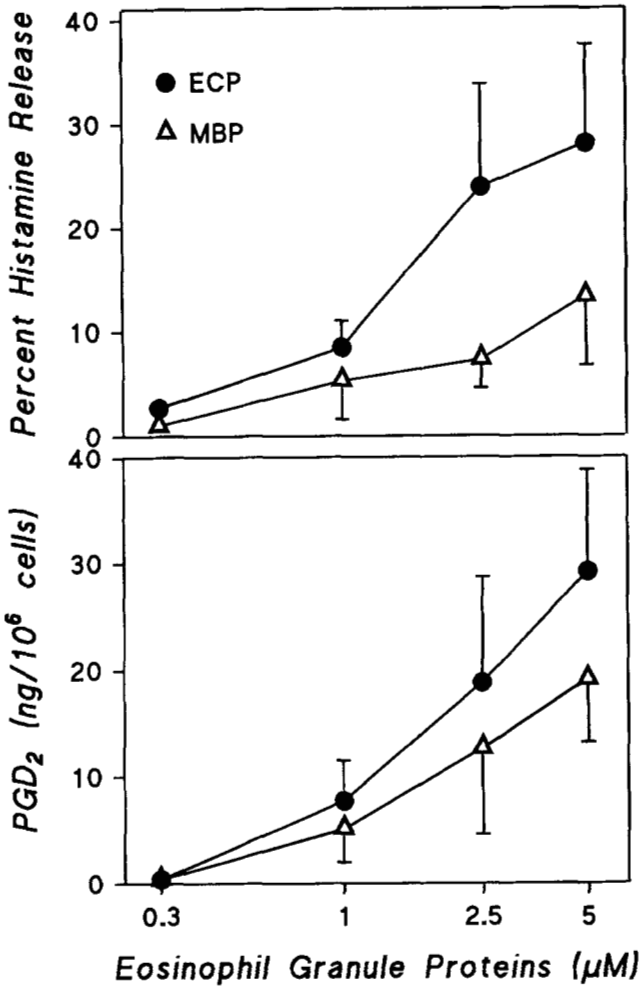

FIGURE 6. Effects of increasing concentrations of ECP and MBP on the release of histamine and the de novo synthesis of PGD_2 by HHMC. Values are expressed as the mean ± SEM of nine experiments.

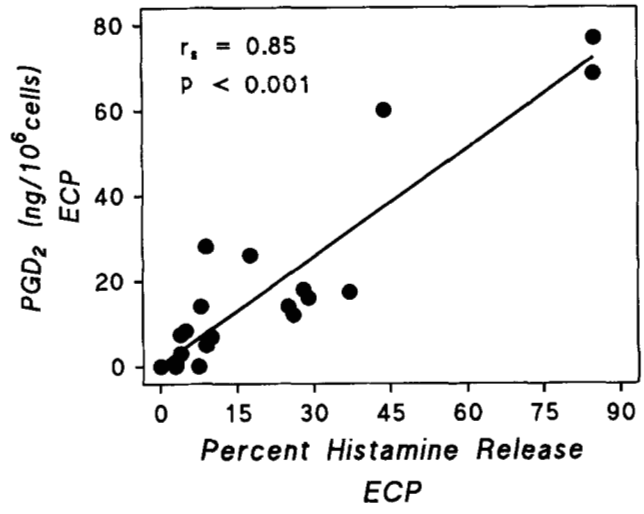

FIGURE 7. Correlation between the maximum percentage of histamine release and PGD_2 synthesis caused by ECP from HHMC. Each point represents the mean of duplicate determinations from separate experiments.

Discussion

This study demonstrates that two eosinophil granule proteins, ECP and, to a lesser extent, MBP, induce the release of preformed and de novo synthesized proinflammatory and vasoactive mediators from mast cells isolated from human heart tissue in a temperature-, energy-, and calcium-dependent manner. HHMC are the only mast cells in man specifically activated by ECP and MBP. This finding is intriguing because eosinophil granule proteins do not induce histamine release from human skin (28) or lung mast cells (L. M. Lichtenstein, unpublished observations). The mechanism for selective activation of HHMC by ECP and MBP is unclear. MBP, but not ECP, induces noncytolytic histamine release from human basophils, whereas MBP and, to a lesser extent, ECP activate rat peritoneal mast cells (26, 27). On the contrary, our findings demonstrate that in humans ECP and, to a lesser extent, MBP activate HHMC, whereas EDN and EPO do not. ECP and MBP are basic polypeptides rich in arginine (53). We have recently demonstrated that protamine, which is also a cationic polypeptide rich in arginine, activates HHMC in vitro (30). However, the ability of ECP and MBP to stimulate the release of preformed and de novo synthesized mediators from HHMC cannot be exclusively explained by net positive charge and high arginine content. In fact, EPO and EDN, at concentrations similar to ECP and MBP, failed to stimulate HHMC. In addition, substance P, another cationic polypeptide that contains arginine, does not activate HHMC (30).

High concentrations of MBP and ECP are cytotoxic for mammalian cells (48–51). However, we show that micromolar concentrations of ECP and MBP activate HHMC in a noncytolytic manner. No detectable LDH release or decrease in cell viability was observed after short-term incubation with ECP and MBP. In addition, the ECP- and MBP-mediated release of preformed and de novo synthesized mediators from HHMC is Ca^{2+}, temperature, and energy dependent. With respect to Ca^{2+} dependence, ECP and MBP caused a greater response at extracellular Ca^{2+} concentrations exceeding 1 mM thus differing from the Ca^{2+} requirement of other immunologic stimuli activating human mast cells (29, 43). However, it is noteworthy that weak stimuli such as platelet-activating factor and IL-3 caused a greater response of

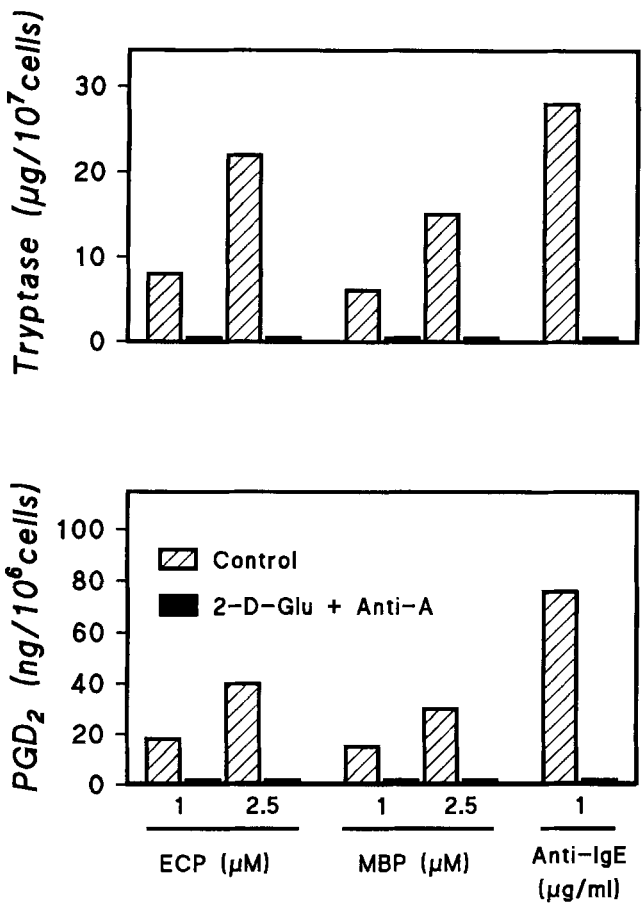

FIGURE 8. Effect of a 15-min cell preincubation with 2-deoxy-D-glucose (2-D-Glu) (10 mM) and antimycin A (Anti-A) (1 μM) on the release of preformed and de novo synthesized mediators from HHMC activated by ECP, MBP, or anti-IgE. Cells were preincubated (15 min, 37°C) with 2-deoxy-D-glucose and antimycin A before the addition of the indicated concentrations of ECP, MBP, or anti-IgE. At the end of incubation (30 min, 37°C), cells were centrifuged and supernatants were analyzed for tryptase and PGD_2. Each bar represents the mean of duplicate determinations from a typical experiment of three performed with similar results.

human basophils at extracellular Ca^{2+} concentrations exceeding 1 mM (54, 55).

Interestingly, in accordance with Thomas et al. (56), we found a significant correlation between the maximum percent histamine release induced by anti-IgE and ECP or MBP. Thus, both basophils and HHMC that respond well to IgE-mediated activation show a significant response to some eosinophil cationic proteins. Although it is unlikely that these correlations are due to interactions between ECP and the IgE-FcεRI network on HHMC, they suggest some similarities in the biochemical steps underlying the release by ECP and by IgE-mediated stimuli.

Given the biologic importance of mast cell-derived mediators (25) in heart pathophysiology (57–60), our findings might explain how eosinophil granule proteins exert tissue damage in the heart of patients with eosinophilia. ECP and MBP deposits were documented within the necrotic and thrombotic lesions found in areas of tissue damage and in the wall of blood vessels (9, 10), and mast cells were present within human heart tissue (29), perivascularly (61), and in the intima of coronary arteries (62). Therefore, activated eosinophils infiltrating human heart tissue may contribute to specific damage through the release of ECP and MBP that in turn can stimulate the secretion of vasoactive and proinflammatory mediators from HHMC.

Tryptase, a neutral protease present in the cytoplasmic granules of human mast cells (46, 47), can activate complement, leading to the formation of anaphylatoxins (C3a and C5a) (63). C5a receptors are present on HHMC and their engagement by C5a leads to HHMC activation and the release of proinflammatory mediators (29, 30, 61). Therefore, the release of tryptase caused by ECP and MBP from HHMC might contribute, as an amplification factor, to the pathogenesis of endomyocardial damage caused by eosinophil granule proteins in patients with persistent or transient eosinophilia.

ECP is released in vivo in anaphylactoid reactions (64) and during Ag challenge in vivo (65). MBP also has been found deposited on the damaged respiratory epithelium (48, 66). The possibility cannot be excluded that ECP and MBP released in vivo during anaphylactic and severe allergic reactions might activate HHMC thus contributing to the cardiovascular derangements observed in some of these patients (67).

Although we obtained similar results with partially purified HHMC (≈12% pure) and unpurified preparations of cardiac mast cells, we cannot exclude the possibility that mediator release from HHMC by ECP and MBP may be influenced by another cell-derived intermediate rather than by direct interaction of eosinophil cationic proteins with cardiac mast cells. Moreover, there was no difference in response in vitro to ECP and MBP between HHMC obtained from donors affected by cardiomyopathy and from subjects without cardiovascular disease who died in car accidents.

In conclusion, we provide the first evidence that ECP and MBP are the only stimuli acting uniquely on human cardiac mast cells as compared with other human mast cells. Moreover, this is the first study to demonstrate that two eosinophil granule cationic proteins (ECP and MBP) selectively activate HHMC to release preformed and de novo synthesized mediators. These findings indicate another mechanism by which infiltrating eosinophils and their proinflammatory mediators contribute to cardiac damage in patients with marked eosinophilia.

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