HISTAMINASE RELEASE FROM HUMAN GRANULOCYTES*

By ROBERT S. ZEIGER,‡ FRANK J. TWAROG,§ AND HARVEY R. COLTEN‖

(From the Division of Allergy, Department of Medicine and Ina Sue Perlmutter Cystic Fibrosis Center, Children's Hospital Medical Center and the Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115)

Histaminase is a specific amine oxidase that deaminates histamine and may be distinguished from the diamine oxidases that catabolize putrescine and cadaverine (1). Histaminase and histamine methyltransferase are the principal, if not the only, enzymes that degrade histamine in man (2), although their relative functional and physiologic importance is uncertain. A histamine-catabolizing enzyme system with physicochemical and functional characteristics of authentic histaminase is found in the granule-rich fraction of human neutrophils and eosinophils; while histamine methyltransferase is in the cell sap of human monocytes (3).

Intact viable polymorphonuclear leukocytes and monocytes exposed in vitro to phagocytic and several other stimuli, release granule-associated lysosomal enzymes (beta glucuronidase, myeloperoxidase, and acid phosphatase (4, 5) and nonlysosomal enzymes such as lysozyme (4) and collagenase (6). Lysosomal enzyme release occurs by exocytosis, whereby the granular membrane and plasma membrane fuse and the contents of the secretory granule are discharged extracellularly (7). Lysosomal enzyme release from granulocytes and histamine release from mast cells and basophils have been shown to be modulated by intracellular levels of cyclic nucleotides (8, 9), metabolic inhibitors (10, 11), and by agents that disrupt or promote aggregation of microtubules (10, 11).

Particles phagocytized by leukocytes, including zymosan coated with complement (4), latex particles bound with IgG (12), aggregated IgG (13), and antigen antibody complexes (4, 12) induce release of lysosomal enzymes. The secretory phase of granulocyte enzyme release may be isolated from phagocytosis and analyzed separately with the use of cytochalasin B (CB)[1] (14) or calcium ionophore (15). CB prevents phagocytosis but facilitates the secretory release of lysosomal enzymes from granulocytes (10, 13). The calcium ionophore A-23187 binds to cell membranes and chelates calcium, thereby promoting calcium influx (16) which may account for its capacity to induce secretion of hormones

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[1] *Abbreviations used in this paper:* CB, cytochalasin B; DMSO, dimethylsulfoxide; LDH, lactic dehydrogenase; K_I, concentration of inhibitor required for 50% inhibition of histamine release.

(insulin) (17), enzymes (amylase) (18), and mediators (histamine, ECF-A) (19, 20). Lysosomal enzymes apparently are released minimally by ionophore (5).

In this report evidence is presented that opsonized zymosan or the calcium ionophore A-23187 induced the release of histaminase from normal and CB-treated human granulocytes. Release was specific, noncytotoxic, dose dependent, and suppressed by metabolic inhibitors and colchicine.

Materials and Methods

Materials. The following were purchased: *Saccharomyces cerevisiae* yeast (zymosan A) (Sigma Chemical Co., St. Louis, Mo.), calcium ionophore A-23187 (Eli Lilly & Co., Indianapolis, Ind.), and CB (I.D.I. Macclesfield, Cheshire, England). CB and calcium ionophore were dissolved in dimethylsulfoxide (DMSO) and diluted for use (final maximal concentration of DMSO, 0.5%). This concentration of DMSO when added to control cells did not alter cell morphology or affect enzyme release or measurement. Phenolphthalein glucuronic acid (Sigma Chemical Co.), histamine dihydrochloride (Sigma Chemical Co.), iodoacetamide (Sigma Chemical Co.), colchicine (Sigma Chemical Co.), lanthanum chloride (Sigma Chemical Co.), sodium azide (Fisher Scientific Co., Fairlawn, N. J.), 2-deoxy-D-glucose (Pfansteihl Labs., Inc., Waukegan, Ill.), aminoguanidine (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.), Hypaque (Winthrop Laboratories, New York), and Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), were obtained in reagent grade. The Tris buffer used was 0.025 M Tris, pH 7.35, at 37°C (Sigma Chemical Co.), 0.12 M sodium chloride, 0.005 M potassium chloride, and 0.3 mg/ml human salt-poor serum albumin (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.). This constitutes Tris-A; Tris ACM contains, in addition, calcium at 0.6 mM and magnesium at 1.0 mM (21). Ring [2-^{14}C]histamine (58 mCi/mmol) (Amersham/Searle Corp., Arlington Heights, Ill.) was repurified by thin-layer chromatography (22).

Methods

PREPARATION OF LEUKOCYTES. 20 ml of peripheral blood anti-coagulated with sodium EDTA (final concentration 5×10^{-3} M) was diluted with an equal volume of Tris A buffer and centrifuged at 400 *g* for 30 min and 10°C on a 15 ml cushion of Ficoll-Hypaque (23). Purified granulocyte suspensions (<2% mononuclear cells) were separated from erythrocytes in the pellet by one to two cycles of hypotonic lysis (3). Mononuclear cells (<1% granulocytes) containing basophils, lymphocytes, and monocytes, were obtained from the interface of the gradient and used for histamine-release experiments.

CELL INCUBATION AND ENZYME-RELEASE PROCEDURES. Leukocyte suspensions containing 0.5-1.0 $\times$ 10^7 cells in 0.10 ml Tris ACM were mixed with varying concentrations of zymosan and calcium ionophore in a final vol of 0.125 ml. Incubation was performed for 30 min at 37°C with intermittent shaking. The reaction was stopped by rapid cooling to 0°C followed by pelleting the cells at 600 *g* for 10 min at 4°C. The supernatant solutions were decanted, frozen at −90°C, and later used for enzyme and histamine determinations. Control buffer blanks were used in each experiment.

VIABILITY OF CELLS. Cell viability at the end of each experiment was assessed by either trypan blue exclusion (Grand Island Biological Co., Grand Island, N. Y.) or lactic dehydrogenase (LDH) release (24). Only experiments in which greater than 90% of the cells remained viable by these criteria are reported.

OPSONIZATION OF ZYMOSAN. Opsonized zymosan was generated by incubating 10 mg of boiled washed zymosan with 1 ml fresh serum from the leukocyte donor for 30 min at 37°C. The opsonized zymosan was washed (five times) in Tris buffer to remove nonadherent serum components. Zymosan to cell ratio varied from 0.25 to 3.0 particles per cell depending on the experimental design.

ENZYME ASSAYS. Histaminase was determined by radioenzyme assay as described previously (22). Briefly, 37.5 ng (2.5 μl) repurified ring [2-^{14}C]histamine were added to 25 μl of supernate or cell lysate and the mixture incubated at 37°C for 5 h. The reaction was terminated by the addition of a 2.5 μl solution containing aminoguanidine (10^{-3} M) and imidazole acetic acid (0.75 mg/ml). For background control the aminoguanidine solution was added to the supernates before the incubation to inhibit the reaction completely at zero time. 15-μl aliquots of the reaction mixture

were then chromatographed 10 cm on cellulose chromatogram plastic back sheets (Eastman Kodak Co., Rochester, N. Y.) using butanol:concentrated acetic acid:water (4:1:1) as solvent. The area containing histamine degradation products was cut and its radioactivity measured by scintillation spectrometry.

Beta glucuronidase was determined on 50 μl of the reaction supernatant solution or 30 μl of the cell lysate with phenolphthalein glucuronic acid as substrate (0.002 M final concentration) and incubated for 18 h at 37°C using the method of Talay et al. (25). LDH was determined using the Dade UV-10-LDH kit (Dade Div., American Hospital Supply Corp., Miami, Fla.) by a modified Wacker method (26) using a Unicam SP 800 A spectrophotometer (Unicam Instruments Ltd., Cambridge, England) at 340 nm.

The total enzyme content (completes) was measured on the supernatant solution obtained after incubating 400 μl of untreated cell suspensions in 10 μg/ml calcium ionophore or 0.1% Triton X-100 (Packard Instrument Co., Inc., Downers Grove, Ill.) for histaminase and only Triton for beta glucuronidase and LDH determination. Incubation time was 30 min at 37°C followed by five freeze-thaw cycles. Cell debris was removed by centrifugation at 10,000 *g* for 5 min at 4°C. Duplicate enzyme assay varied by no more than 10%. The sum of residual cellular enzyme activity and release enzyme activity approached 100%.

HISTAMINE ASSAY. Leukocyte histamine was determined by the method of May et al. (27). The total histamine content was estimated on a portion of the cells after boiling.

Results

Zymosan-Induced Histaminase Release from Human Granulocytes. Purified human granulocytes were incubated with varying concentrations of opsonized zymosan for 30 min at 37°C[2] and the histaminase and beta glucuronidase released into the medium were determined. Enzyme release was a function of zymosan concentration from 0.25 to 3.0 particles per cell (Fig. 1). The highest particle concentration released virtually all the cellular histaminase while only releasing 20% of the beta glucuronidase (Fig. 1). In other experiments the maximum histaminase released by three particles per cell varied from 60 to 100% depending on the granulocyte preparation; moreover, the percentage of histaminase released was characteristically at least four to five times greater than that of beta glucuronidase. Histaminase release was maximal at 37°C, completely prevented at 4°C, and was only 5 and 30% of maximal at 20°C and 30°C, respectively. Release occurred without the concomitant release of LDH or the uptake of trypan blue, indicating a noncytotoxic release of the enzyme.

Kinetics of Zymosan-Induced Histaminase Release. Granulocytes prewarmed to 37°C were mixed with opsonized zymosan, the reaction mixtures were rapidly cooled to 0°C at timed intervals, and the cells pelleted by centrifugation. Preliminary experiments indicated that this procedure prevented further enzyme release. As shown in Fig. 2, histaminase release began after a period of 2–5 min, and was maximal at about 30 min. A similar time-course was noted for beta glucuronidase release, however, at each particle concentration, release of this enzyme was only 20–25% that of histaminase release.

Effect of Opsonization on Zymosan-Induced Granulocyte Histaminase Release. Human granulocytes incubated with opsonized zymosan at a concentration of one particle per cell released 55% of the total cellular histaminase content. Spontaneous release was 13% (Fig. 3). Cells incubated with zymosan alone or with zymosan pretreated with heat-inactivated serum (56°C, 1 h) released significantly less histaminase than cells exposed to opsonized particles.

[2] These conditions were chosen on the basis of the kinetic experiments described below.

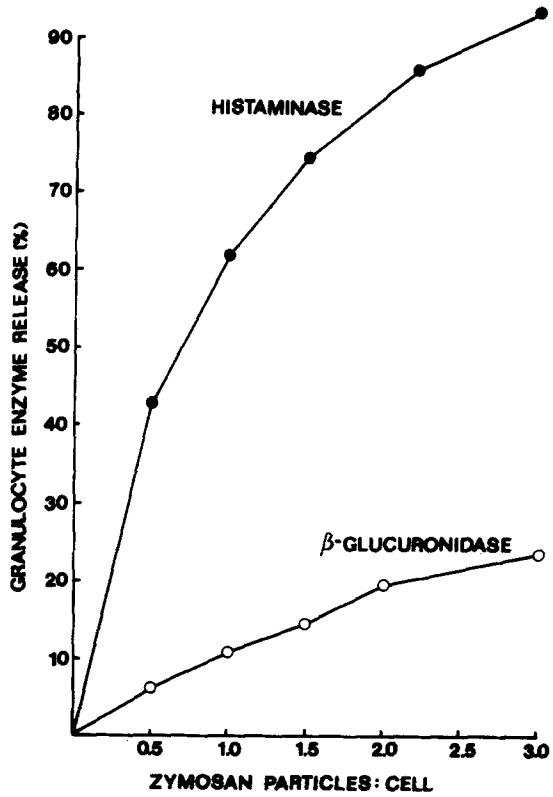


FIG. 1. Release of histaminase and β-glucuronidase from human granulocytes induced by opsonized zymosan: effect of particle/cell ratio. Each point represents the mean net release from duplicate incubation mixtures. Total histaminase activity, 100.6 pmol/h; β-glucuronidase, 28.7 μg phenolphthalein/h/0.5 × 10^7 granulocytes.

Light microscopy demonstrated that opsonization also potentiated the phagocytosis of zymosan particles. Beta glucuronidase release was maximal with opsonized zymosan but total release was less than 10% at the particle to cell concentration used in these experiments.

Effect of Divalent Cations on Histaminase Release. The requirement of divalent cations for opsonized zymosan-induced histaminase release is shown in Fig. 4. Granulocyte histaminase release induced by opsonized zymosan was maximal in the presence of both calcium and magnesium. Optimal release occurred at a calcium concentration of 0.6 mM and magnesium concentration of 1.0 mM. Specific histaminase release was markedly reduced when either calcium or magnesium were present alone and was prevented with the absence of added divalent cations or in the presence of 1 × 10^{-3} M EDTA; lanthanum chloride (10^{-4} M), an agent that interferes with calcium flux (28), inhibited opsonized zymosan-induced histaminase release (Table I). Beta glucuronidase release was similarly dependent on divalent cations but again was less than 10% at the particle to cell concentration used.

Effect of CB on Zymosan-Induced Histaminase Release. Preliminary experiments demonstrated that purified human granulocytes preincubated with CB

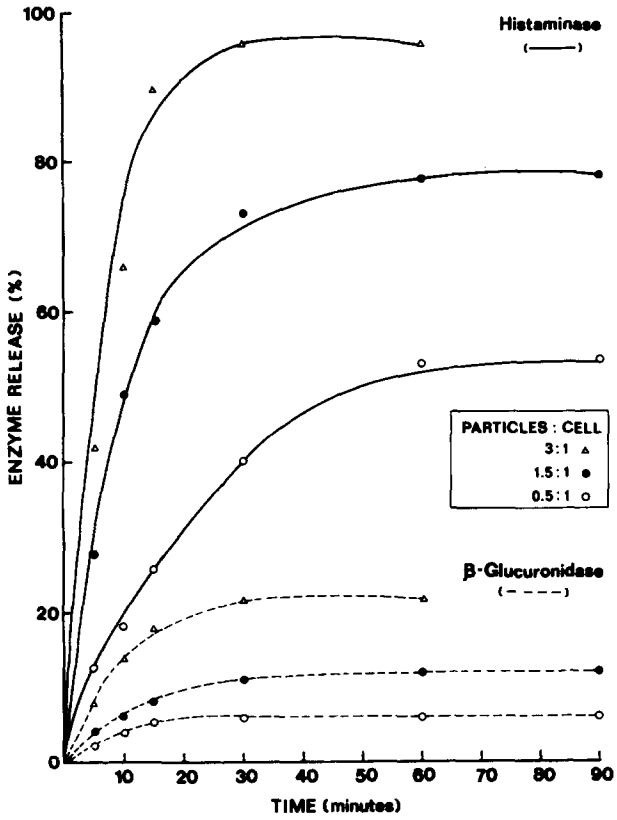


FIG. 2. Kinetics of histaminase and β-glucuronidase release from human granulocytes. Zymosan was incubated with granulocytes at 37°C in duplicate and the reaction terminated at the indicated times. Total histaminase activity, 111 pmol/h and β-glucuronidase, 41.5 μg phenolphthalein/h/0.9 × 10^7 cells.

(5 μg/ml) for 20 min at 20°C failed to phagocytize opsonized zymosan particles but did release substantial quantities of histaminase. As presented in Fig. 3, granulocytes pretreated with CB at concentrations of 5–10 μg/ml and then incubated with opsonized zymosan released nearly 80% of their histaminase, an amount nearly twice that released in the absence of CB. Zymosan-induced release of histaminase from CB-treated granulocytes was dependent on the presence of calcium and magnesium (Fig. 4). Beta glucuronidase release was also enhanced after CB pretreatment and was dependent on divalent cations, in agreement with previous reports (8).

Calcium Ionophore-Induced Histaminase Release from Granulocytes. Suspensions of human granulocytes and mononuclear cells were each incubated with varying concentrations of the ionophore A-23187 for 30 min at 37°C. Release of histaminase, beta glucuronidase, and LDH from granulocytes and histamine from the basophil-rich mononuclear cells was then determined. The extent of histaminase release from granulocytes and histamine release from the mononuclear cell fraction were functions of the ionophore concentration between 0.20 and 1.0 μg/ml (Fig. 5). Ionophore-induced histaminase release was abrogated in

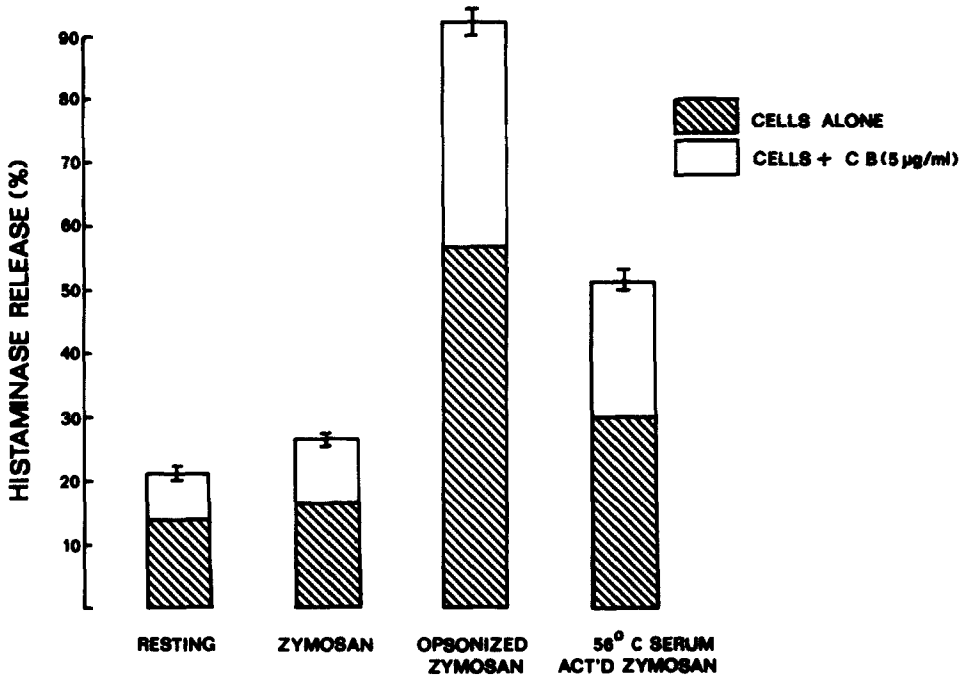
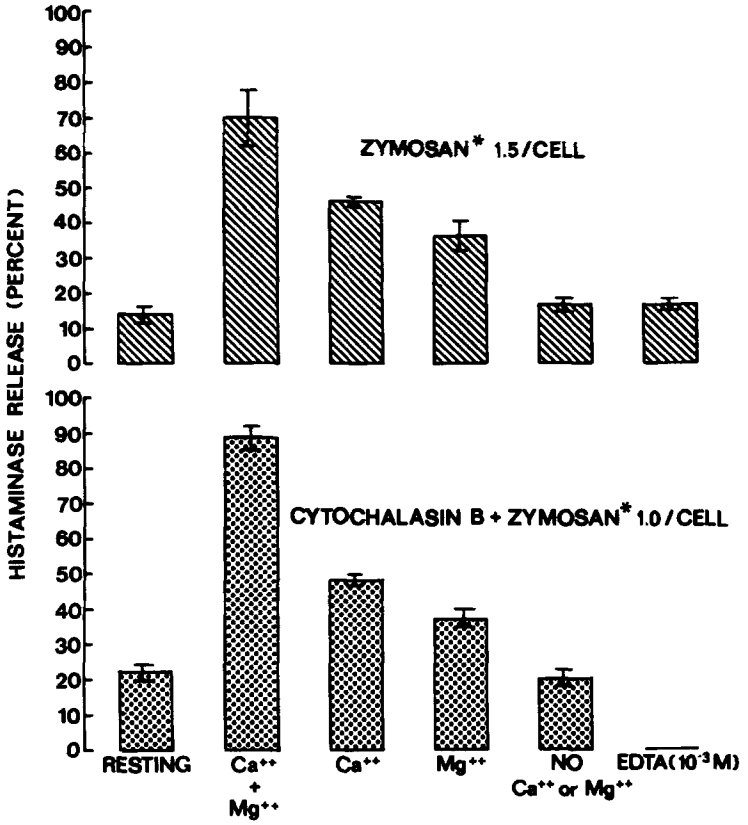


FIG. 3. Effect of opsonization on zymosan-induced release of granulocyte histaminase. Boiled zymosan (10 mg) opsonized by incubation with fresh human serum (1 ml) 30 min at 37°C. Controls included zymosan plus heat-inactivated (56°C, 1 h) serum and zymosan alone. Each zymosan preparation was added to either CB-pretreated (5 µg/ml) or untreated granulocytes. Values expressed as the mean enzyme released from triplicate incubation mixtures. Total histaminase activity, 140 pmol/h/0.5 × 10^7 cells.

the absence of calcium ions. Lanthanum chloride inhibited ionophore-induced release in dose-dependent fashion (Table I). Magnesium was unable to substitute for calcium. Less than 5% beta glucuronidase and no LDH was released by the calcium ionophore even at high ionophore concentrations. As presented in Fig. 6, the kinetics of calcium ionophore-induced granulocyte histaminase release were similar to the kinetics of zymosan-induced release (Fig. 2).

Effect of Metabolic Inhibitors and Colchicine on Granulocyte Histaminase Release. Aliquots of a granulocyte suspension were preincubated with varying concentrations of inhibitor for 15 min at 37°C and then opsonized zymosan or calcium ionophore A-23187 were added. Controls consisted of cells incubated with the metabolic inhibitors but without zymosan or ionophore; and cells incubated in the absence of the inhibitors but with the appropriate releasing agents. An identical experiment was performed except that the effect of metabolic inhibitors was examined in the presence of CB (5 µg/ml added with the inhibitors). A K_I (concentration of inhibitor required for 50% inhibition of histaminase release) was calculated from the dose response curves for each inhibitor. Preliminary experiments indicated that the metabolic inhibitors had no effect on the assay for histaminase activity. Deoxyglucose, sodium azide, and iodoacetamide each at concentrations of 10^{-3} M or less significantly inhibited histaminase release (Table II).



IG. 4. Effect of divalent cations on granulocyte histaminase release. Values expressed as ean ± 1 SD of triplicate determinations. Total histaminase activity, 120 pmol/h/0.5 × 10^7 lls.

TABLE I

Inhibition of Histaminase Release from Human Granulocytes by Lanthanum Chloride ($LaCl_3$) *

Concentration of $LaCl_3$	Zymosan induced‡		Ionophore induced§	
	Release	Inhibition	Release	Inhibition
M	%	%	%	%
10^{-4}	11.5	40	0	100
10^{-5}	14.3	26	51.0	43
10^{-6}	19.0	0	65.4	27
10^{-7}	19.4	0	90.0	0
None	19.3		90.2	

* Granulocyte suspensions in Tris A buffer preincubated with $LaCl_3$ for 10 min at 4°C followed by addition of calcium (0.6 mM final concentration) were then exposed to zymosan or ionophore for 30 min at 37°C. Results expressed as the mean release from duplicate incubation mixtures.

‡ Opsonized zymosan (0.5 particle per cell); total histaminase activity, 168 pmol/h/0.56 × 10^7 cells.

§ Ionophore (0.8 μg/ml); total histaminase activity, 107 pmol/h/0.57 × 10^7 cells.

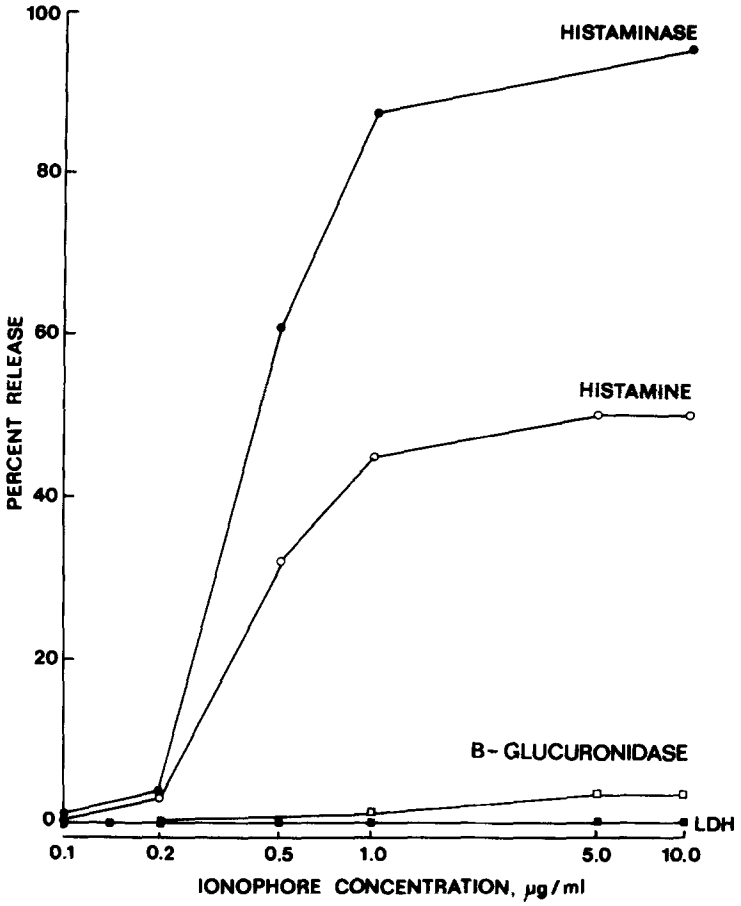


FIG. 5. Ionophore-induced histaminase and histamine release. Granulocytes and mononuclear cells exposed separately to varying concentrations of ionophore at 37°C for 30 min. Enzyme release from the granulocytes and histamine release from the mononuclear cells determined in duplicate. Total histaminase activity, 85.4 pmol/h; β-glucuronidase, 42.5 μg phenolphthalein/h; and LDH, 907 Wacker U/min (37°C)/0.8 × 10^7 granulocytes. Total histamine content, 0.18 μg/0.5 × 10^7 mononuclear cells.

A similar experiment designed to test the effect of colchicine on opsonized zymosan or ionophore-induced histaminase release demonstrated inhibition of release with K_I of 5 × 10^{-4} M (Table II). Further evidence of a role for microtubules in histaminase release was obtained in an experiment showing a 30% enhancement of zymosan-induced release in the presence of buffer containing heavy water (D_2O, 44%).

Discussion

Equipped with histamine catabolic enzymes (3), human leukocytes possess the potential to inactivate histamine at sites of inflammation. The present studies were designed to evaluate the capacity of granulocytes to release histaminase in response to both phagocytic and secretory stimuli. The experiments demonstrated that human granulocytes released histaminase quantitatively

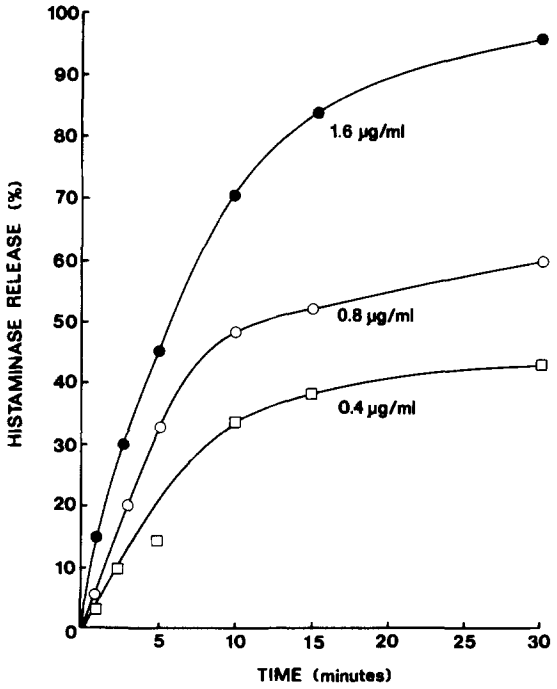


FIG. 6. Kinetics of ionophore-induced histaminase release from human granulocytes: effect of ionophore concentration. Values expressed as the mean of duplicate incubation mixtures. Total histaminase activity, 255 pmol/h/0.75 × 10^7 cells.

TABLE II
Inhibition of Histaminase Release from Human Granulocytes

Inhibitor*	K_i‡		
	Zymosan induced§		Ionophore induced‖
	Cells alone	Cells with CB¶	Cells alone
Iodoacetamide	0.20	0.50	1.00
2-Deoxyglucose	0.45	>1.0**	0.40
Sodium azide	0.10	ND‡‡	ND
Colchicine	0.50	0.50	1.00

* Inhibitors preincubated with cells for 15 min at 37°C; also present during the 30-min exposure to zymosan or ionophore.

‡ K_I, concentration (mM) yielding 50% inhibition of release.

§ Opsonized zymosan (1.5 particles per cell) released 51 and 90% granulocyte histaminase from normal and CB-treated cells, respectively. Total histaminase activity, 72.8 pmol/h/0.5 × 10^7 cells.

‖ Ionophore (1 µg/ml) released 91% of cellular histaminase. Total histaminase activity, 140 pmol/h/1.0 × 10^7 cells.

¶ CB (5 µg/ml) preincubated with cells for 15 min at 37°C.

** 0–20% inhibition at 1 mM.

‡‡ ND, not determined.

when incubated in vitro with either opsonized zymosan or the calcium ionophore A-23187. Histaminase release required the presence of divalent cations (both calcium and magnesium for optimal release after opsonized zymosan and calcium alone for ionophore-induced release). Release was inhibited by EDTA, a chelating agent, and by lanthanum chloride, an agent which interferes with transmembrane movement of calcium ions (29).

The necessity of energy generation for histaminase release was indicated both by the temperature dependence of release and the suppression of release by the metabolic inhibitors 2-deoxyglucose, iodoacetamide, and sodium azide. The involvement of microtubules in histaminase release was suggested by the studies which demonstrated inhibition of release by colchicine and potentiation of release by D_2O. Granulocyte histaminase release appeared noncytotoxic since release was not accompanied by either the concomitant uptake of trypan blue or the release of the cytoplasmic enzyme LDH. Preliminary studies suggest a similar pattern of release of histaminase from human eosinophils stimulated with opsonized zymosan, latex particle, or calcium ionophore.

These results suggest a noncytotoxic stimulus-coupled release of histaminase from human granulocytes. Weissmann et al. (4) have shown that lysosomal enzymes (beta glucuronidase and acid phosphatase) and lysozyme were released from granulocytes challenged with zymosan, while Goldstein et al. (5) demonstrated that lysozyme was released by ionophore. The potentiation of histaminase release by opsonization of particles was in agreement with the studies of others (4, 12). Opsonized zymosan-induced histaminase release approached 100% during optimal particle contact, while beta glucuronidase release reached only 25%. Moreover, histaminase release induced by opsonized zymosan, occurred at particle to cell ratios which resulted in only minimal beta glucuronidase release. Ionophore stimulated a dose-dependent release of granulocyte histaminase which approached 100% at concentrations of ionophore that barely released beta glucuronidase.

It has been suggested that differences in granulocyte enzyme release may relate to the physical and chemical properties of the limiting membranes of the particular class of granule (28). Although histaminase has been localized to the granule-rich fraction of human granulocytes, specific granule localization is not yet known. Lysozyme-containing granules in the human granulocyte apparently have been shown to be distinct from the azurophilic granules which contain lysosomal enzymes, such as beta glucuronidase and myeloperoxidase (30). The release experiments suggest that histaminase may therefore reside in a granule distinct from the lysosomes.

The augmentation of opsonized zymosan-induced histaminase release by CB was in agreement with the potentiation by CB of release of granulocyte enzymes as noted by others (5, 12). Goldstein et al. have suggested that recognition of C5a may be important for CB-induced release of lysosomal enzymes and lysozyme (31).

The modulation of allergic reactions by leukocyte products has received recent support. Wasserman et al. have shown that eosinophil arylsulfatase inactivates SRS-A (32) and Goetzl et al. demonstrated that phagocytosis by guinea pig eosinophils of starch particles resulted in the release of arylsulfatase (33). Kater

et al. (34) have demonstrated that eosinophils possess a phospholipase D which can inactivate mast cell and basophil platelet-aggregating factor. Studies by Hubscher (35) suggested that prostaglandins derived from human eosinophils are capable of inhibiting antigen-triggered histamine release. The presence of a histaminase catabolic enzyme system in human neutrophils and eosinophils which is released in response to appropriate stimuli provides evidence that granulocytes may modulate hypersensitivity reactions by inactivating histamine at sites of inflammation.

Summary

Histaminase (EC-1.4.3.6), one of the two catabolic enzymes for histamine, is contained in human granulocytes. Opsonized zymosan or the calcium ionophore A-23187 induce a dose-dependent release of histaminase from human granulocytes in vitro. Release is completed within 30 min, is temperature dependent, and requires divalent cations. Opsonized zymosan-induced histaminase release was maximal in the presence of both calcium and magnesium, whereas ionophore release was magnesium independent. The total cellular content of histaminase could be released by both opsonized zymosan and ionophore. In contrast, only 25% of the cellular beta glucuronidase, a lysosomal enzyme, was released after maximal stimulation with opsonized zymosan; there was minimal release of beta glucuronidase with ionophore. Zymosan- and ionophore-induced histaminase release was inhibited by agents that are presumed to interfere with cell metabolism and disrupt microtubules. Human granulocytes therefore may modulate the effect of histamine by releasing histaminase at a site of inflammation. Studies of granulocyte histaminase release in vitro may also provide a new model to explore granulocyte function and secretion.

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