Inhibition of Histaminase Release from Human Granulocytes by Products of Histaminase Activity

Abstract. *Imidazoleacetic acid, a product of the action of histaminase (E.C. 1.4.3.6) on histamine, inhibits specific release of histaminase from human peripheral blood granulocytes with an inhibition constant between 5 × 10⁻⁹M and 1 × 10⁻⁸M. Hence, modulation of enzyme release is indirectly mediated by the activity of the enzyme.*

Complex control mechanisms have been described for mediators of inflammation (*1*). Several such mechanisms depend on enzymes that have the capacity to inactivate products of mast cells or basophils. Slow-reacting substance of anaphylaxis (SRS-A), platelet activating factor, and histamine are inactivated by enzymes contained in peripheral blood leukocytes (*2, 3*).

Histamine catabolism in humans is accomplished by the action of two enzymes, histamine methyltransferase (E.C. 2.1.1.8) (*4*) and histaminase (E.C. 1.4.3.6) (*5*). In peripheral white blood cells, histamine methyltransferase is contained exclusively in monocytes and histaminase, in the granulocytes. Leukocyte histaminase is found in a granule-rich fraction after differential centrifugation of cell lysates (*3*). Noncytotoxic dose-dependent release of histaminase is induced by a fragment of the third complement component (C3b) when C3b is bound to zymosan, erythrocytes, or Sephadex G-75 (*6*). Granulocyte histaminase release induced by washed opsonized zymosan or calcium ionophore (A23187) is inhibited by agents that interfere with cell metabolism and disrupt microtubules. Concentrations of bound C3b that induce significant granulocyte histaminase release result in only minimal release of β-glucuronidase (*6*), even in the presence of cytochalasin B, an agent that blocks phagocytosis, but enhances secretory release of lysosomal enzymes (*7*).

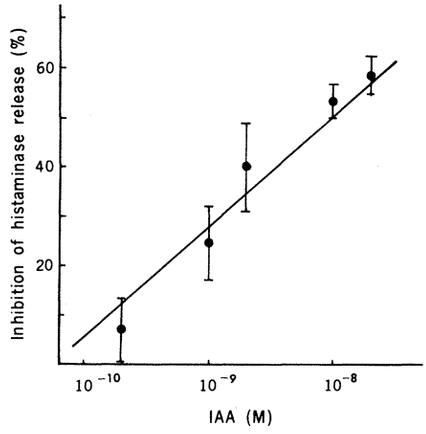

Fig. 1. The effect of IAA on opsonized zymosan-induced granulocyte histaminase release. Human neutrophils (0.5 × 10⁷ in 0.15 ml of tris-ACM) were first incubated with various concentrations of IAA for 20 minutes at 4°C; then, without being washed, they were incubated with opsonized zymosan (2.5 particles per cell) for 30 minutes at 37°C, and histaminase release was measured. Results are expressed as the percentage of inhibition of histaminase release from control, and represent the mean ± 1 standard deviation (S.D.) of five separate experiments. Control release was 58.1 ± 9.4 percent and spontaneous release, 12.8 ± 2.0 percent; total neutrophil histaminase was 106.3 ± 14.3 pmole per hour per 0.5 × 10⁷ neutrophils.

Histaminase effects catabolism of histamine by way of imidazole-4-acetaldehyde (an unstable intermediate) to imidazoleacetic acid (IAA), the bulk of which is then excreted in urine, free or conjugated to ribose. In preliminary experiments, opsonized zymosan-induced histaminase release from human granulocytes was inhibited in the presence of metabolites generated by the action of histaminase on histamine (*8*). Accordingly, a detailed study of the effect of IAA and related compounds on histaminase release was undertaken.

Partially purified granulocyte suspensions were obtained by centrifugation of whole blood (*9*) on Ficoll-Hypaque cushions and then subjecting the red blood cells to hypotonic lysis. Granulocyte suspensions (< 2 percent mononuclear) containing 0.5 × 10⁷ cells in a final volume of 0.15 ml of tris-ACM (*10*) were first incubated for 20 minutes at 4°C (*11*) with varying concentrations of histamine metabolites, imidazole compounds, other amines, or buffer. Opsonized zymosan (2.5 particles per cell) was then added to the cell suspensions and the mixtures were incubated for 30 minutes at 37°C with intermittent shaking. The enzyme release reaction was terminated by rapidly cooling the mixtures to 4°C; the cell suspensions were then centrifuged (600*g* for 10 minutes at 4°C) and the supernatant solutions were decanted, frozen at −90°C, and used later for enzyme determinations. Total cellular histaminase was determined in cells to which an excess of calcium ionophore (A23187) was added. Histaminase was measured by a radiochromatographic assay, as previously described (*12*). Histaminase activity varied by less than ± 10 percent in duplicate samples and by less than ± 5 percent in replicate determinations. Only experiments in which greater than 90 percent of the cells remained viable by Trypan blue exclusion are reported.

Imidazoleacetic acid at molar concentrations of 3 × 10⁻¹⁰ to 3 × 10⁻⁸ inhibited opsonized zymosan-induced histaminase release from granulocytes (Fig. 1). The extent of inhibition was found to be a function of IAA concentration. Imidazoleacetic acid had no effect on the histaminase assay at concentrations of $10^{-4}M$ or less and did not induce enzyme release in the absence of opsonized zymosan. The inhibition constant (K_i) of IAA, that is, the concentration sufficient to yield 50 percent inhibition of histami-

Table 1. Modulation of complement-induced histaminase release from human granulocytes. The compounds were incubated with cells for 20 minutes at 4° or 25°C as described in text and then, during exposure to opsonized zymosan (2.5 particles per cell), for 30 minutes at 37°C. Supernatants were assayed for histaminase release. Control release (opsonized zymosan alone) was 46 ± 8.2 percent (mean ± S.D.) and spontaneous release was 12.6 ± 3.2 percent for 12 experiments; total granulocyte histaminase content was 114 ± 15.7 pmole per hour per 0.5 × 10⁷ neutrophils. Results expressed as mean percentage of inhibition of histaminase release in five separate experiments for IAA, 1-methyl-4-imidazoleacetic acid, histidine, theophylline, and isoproterenol; three separate experiments for histamine, isoproterenol + 3-isobutyl-1-methylxanthine (MIX), dibutyryl cyclic AMP, cyclic GMP, propranalol, carbachol, papaverine, and atropine; and the average of duplicate experiments for the other inhibitors. Isoproterenol ($10^{-6}M$) + MIX ($10^{-5}M$) produced 48.6 ± 3.2 percent inhibition and 1-methyl-4-imidazoleacetic acid ($10^{-4}M$), 50.4 ± 7.1 percent inhibition. Where K_i is indeterminate and designated "greater than," higher concentrations of the agents were not tested because they interfered with the histaminase assay. The K_i values indicate the micromolar concentrations causing 50 percent inhibition of histaminase release.

Compound	K_i
Imidazoleacetic acid	0.005 to 0.01
1-Methyl-4-imidazole-acetic acid	100
Imidazole	> 100
Urocanic acid	> 100
Histidine	> 100
Histamine	> 10
Cadaverine	> 10
Spermine	> 10
Spermidine	> 10
Isoproterenol	> 10
MIX	> 10
Isoproterenol + MIX*	1
Dibutyryl cyclic AMP	> 1000
Propranalol	> 50
Carbachol	> 100
Cyclic GMP	> 10
Papaverine	> 10
Atropine	> 10

*3-Isobutyl-1-methylxanthine, at 10 μ*M*.

nase release, was between $5 \times 10^{-9}M$ and $1 \times 10^{-8}M$. This effect on enzyme release by IAA was reversible; that is, when granulocytes were washed after incubation with IAA, opsonized zymosan-induced release of histaminase was similar to that released from cells first incubated in buffer alone. Inhibition of release was not dependent on an effect of IAA on phagocytosis (another stimulus for enzyme release) since IAA also inhibited release from cells treated with cytochalasin B at concentrations that block phagocytosis. The IAA had no effect on opsonized zymosan-induced granulocyte release of β-glucuronidase, myeloperoxidase, or lysozyme (data not shown) (*13*).

Inhibition of granulocyte histaminase release by IAA was compared to the effects of other products of histamine catabolism, imidazole compounds, and amines, as shown in Table 1. Histaminase release was inhibited 50 percent by the histamine methyltransferase metabolite, 1-methyl-4-imidazoleacetic acid at $10^{-4}M$ concentration, a K_i 10,000 times greater than the K_i for IAA. The K_i for imidazole, histidine, and urocanic acid were indeterminate, but were more than 10,000 times that for IAA. Histaminase release was inhibited 10 percent or less at the highest concentration of these agents tested. The K_i for histamine, as well as other amines, cadaverine, spermine, and spermidine were greater than 1000 times that for IAA.

Lysosomal enzyme release from granulocytes (*14*) and histamine release from mast cells and basophils are modulated by intracellular concentrations of cyclic nucleotides (*15*). Imidazole enhances mediator release, perhaps because of decreased concentrations of mast cell adenosine 3′,5′-monophosphate (cyclic AMP) (*16*). To test whether agents known to modulate cellular levels of cyclic AMP and guanosine 3′,5′-monophosphate (cyclic GMP) would affect granulocyte histaminase release, we conducted the following experiments. Adrenergic or cholinergic agents, a phosphodiesterase inhibitor, exogenous dibutyryl cyclic AMP and cyclic GMP were each incubated with granulocytes for 20 minutes at 25°C and then during exposure to opsonized zymosan (2.5 particles per cell).

For each agent, at the highest concentrations that could be tested, only minimal inhibition of histaminase release was observed. The K_i was, therefore, indeterminate. However, incubation of granulocytes with isoproterenol in the presence of a phosphodiesterase inhibitor led to inhibition of histaminase release (Table 1). Under these conditions, the K_i for isoproterenol was 100 to 200 times that of IAA.

These data provide evidence for an indirect feedback inhibition by imidazoleacetic acid of histaminase release from granulocytes. Physiological concentrations of IAA, the principle end product of the action of histaminase on histamine, block enzyme release. Related compounds were at least 1000 times less potent inhibitors of histaminase release. Histamine and IAA may play a role in eosinophil chemotaxis (*17*). Since the eosinophils also contain histaminase (*3*), stimuli for release of the enzyme may, therefore, indirectly affect the accumulation of eosinophils at an inflammatory site.

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10. Tris-ACM buffer consisted of 0.025*M* tris, *p*H 7.35 at 37°C, 0.12*M* sodium chloride, 0.005*M* potassium chloride, 0.3 of human salt-poor albumin (Hyland) per milliliter, 0.6 m*M* calcium chloride, and 1.0 m*M* magnesium chloride [L. M. Lichtenstein and A. G. Osler, *J. Exp. Med.* **120**, 507 (1964)].
11. The first period of incubation was at 4°C to limit spontaneous release of histaminase. However, inhibition of enzyme release by IAA was also observed when the mixtures were incubated at 25° or 37°C.
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Heavy Metals Affect Rod, But Not Cone, Photoreceptors

Abstract. *Low concentrations of lead, mercury, or cadmium depress the amplitude of the rod receptor potential in the perfused bullfrog retina. Responses from the cones were not affected. The data implicate the rods as a lesion site in animals exhibiting scotopic vision deficits as a result of heavy metal poisoning.*

Vision deficits in human and nonhuman primates after exposure to either lead (Pb^{2+}) (*1, 2*) or methylmercury (*2, 3*) have been attributed to decreases in the rod-mediated, scotopic, visual system as opposed to the cone-mediated, photopic, visual system. There is little doubt that at least some of the reported deficit is due to a central lesion (*2, 3*). However, some retinal involvement is suggested by a report describing decreases in the amplitude of the b wave of the electroretinogram (ERG) as an early indicator of Pb^{2+} toxicity in occupationally exposed workers (*4*). To determine whether the deficits caused by Pb^{2+} and mercury (Hg^{2+}) involve rod or cone photoreceptors (or both), we studied the isolated, perfused bullfrog (*Rana catesbeiana*) retina. We report here that inorganic Pb^{2+}, Hg^{2+}, and cadmium (Cd^{2+}) affect the rod, but not the cone, photoreceptors.

The dissected retina, minus pigment epithelium, from a dark-adapted bullfrog was positioned in a chamber and perfused (*5*) with Ringer solution containing 100.0 m*M* NaCl, 2.0 m*M* KCl, 5.0 m*M* glucose, 0.4 m*M* $MgCl_2$, 0.4 m*M* $CaCl_2$, 10.0 m*M* sodium aspartate, and 20.0 m*M* tris-maleate buffered to *p*H 7.8. Sodium

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