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Inhibition of human polymorphonuclear leukocyte-derived histaminase activity by H-2 antagonists*

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Histamine is released from sensitized basophils or mast cells upon interaction with appropriate antigen [1]; this process represents one of the main components of the allergic response. Once released, histamine may be metabolized via two alternate pathways, methylation by histamine methyltransferase (*S*-adenosyl-L-methionine: histamine *N*-methyltransferase; EC 2.1.1.8) or oxidative deamination by diamine oxidase [diamine, O_2 oxidoreductase (deaminating); EC 1.4.3.6] [2]. With histamine as the substrate, the latter enzyme is commonly referred to as histaminase. It has recently been reported that human polymorphonuclear leukocytes (PMN) possess histaminase activity [3] and that the enzyme is released during incubation with a phagocytic stimulus or with the calcium ionophore A23187 [4]. The presence of histaminase in PMN and its release during phagocytic stimulation suggest that it may participate in the metabolism of histamine released during immediate hypersensitivity reactions. The likelihood of this possibility is enhanced by evidence that neutrophil chemotactic factors are released from basophils and mast cells [5, 6]. The chemotactic factors would contribute to an accumulation of PMN in the vicinity of the released histamine.

The pro-inflammatory effects of histamine on smooth muscle are well known [2, 7]; more recently appreciated is its role as a negative modulator of inflammatory reactions. Histamine has been reported to inhibit antigen-induced histamine release from human basophils [8], lysosomal enzyme release from human PMN [9], and murine lymphocyte-mediated cytolysis [10]. The action of histamine in each of these systems is blocked by histamine antagonists of the H-2 class but not of the H-1 class [9–11]. Because of possible applications of H-2 antagonists to the regulation of these reactions, as well as the structural similarity of H-2 antagonists to histamine [12], the influence of selected H-2 antagonists on PMN-derived histaminase activity was examined. The results presented here indicate that histamine antagonists of the H-2 class competitively inhibit PMN-derived histaminase activity.

To obtain histaminase, human polymorphonuclear leukocytes were isolated from venous blood of a normal donor by Hypaque–Ficoll density centrifugation [13]. Contaminating erythrocytes were removed by hypotonic lysis and the PMN suspended at 10^7 cells/ml in a 0.025M Tris buffer, pH 7.4, containing 0.3 mg/ml of human serum albumin, 0.6 mM $CaCl_2$, and 1 mM $MgCl_2$ (TACM) [1]. One-ml volumes of the PMN suspension were incubated with 5 μg/ml of A23187 (suspended in buffer) for 30 min at 37°. Zeiger *et al.* [3] demonstrated that A23187 releases all of the intracellular histaminase activity within 30 min and this has been confirmed in our laboratory. Incubations were stopped by a 2-min centrifugation at 1000 *g* and the cell-free supernatant fractions were pooled. The pooled supernatant fraction was diluted 1 : 1 with buffer to yield activity derived from 5×10^6 PMN/ml and divided into aliquots for storage at −20°.

Histaminase activity was measured by the method of Beaven and Shaff [14]. The assay is based on quantitating tritiated water liberated during deamination of [β-^{3}H]-histamine. The assay mixture consisted of 0.05 ml PMN supernatant fraction, 0.05 ml [β-^{3}H]histamine and 0.05 ml of drug solution or 0.1 M phosphate buffer, pH 6.8. Desired concentrations and specific activities of the substrate were prepared by mixing aliquots of stock [β-^{3}H]histamine (11 Ci/m-mole) with unlabeled histamine in the phosphate buffer. The reactions were stopped at indicated times by the addition of 0.1 ml of 0.1 M histamine solution containing 1 g/ml of AG 50W-X8 (H^+). The samples were centrifuged for 5 min at 1000 *g* and an aliquot of the supernatant fraction was counted in a liquid scintillation counter.

Stock solutions (0.1 M) of metiamide and cimetidine were prepared by dissolving the compounds in 1 N HCl and adjusting the solutions to approximately pH 6 by the addition of 0.1 N NaOH and distilled H_2O. Dilutions to desired concentrations were made with the phosphate buffer. Stock solutions (0.01 M or 0.1 M) of remaining drugs were prepared in the phosphate buffer and further dilutions made with the phosphate buffer. All stock solutions were stored at −20°.

The [β-^{3}H]histamine was a generous gift from Dr. Michael Beaven at NIH. The H-2 antagonists were kindly supplied by Dr. Lawrence Chakrin of Smith Kline & French Laboratories (Philadelphia, PA). Diphenhydramine hydrochloride histamine dihydrochloride, imidazole, and imidazole acetic acid hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO), aminoguanidine sulfate from Eastman Kodak (Rochester, NY) and AG 50W-X8(H^+, 100–200 mesh) from Bio-Rad Laboratories (Richmond, CA). Pyrilamine maleate was obtained from Merck & Company, (West Point, PA), chlorpheniramine maleate from Schering Corp., (Kenilworth, NJ) cyclizine hydrochloride from Burroughs Wellcome Co. (Research Triangle Park, NC), and promethazine hydrochloride from Wyeth Laboratories (Philadelphia, PA).

Histaminase activity was linear with time for 18 hr of incubation (Fig. 1, left panel). Activity was completely abolished by 10^{-5} M aminoguanidine, a specific inhibitor of diamine oxidase [14]. A Lineweaver–Burk plot of activity vs substrate concentration yielded a K_m of 2.4×10^{-6} M (Fig. 1, right panel). This value agrees with the K_m of 2.5×10^{-6} M reported by Zeiger *et al.* [3] for histaminase activity of human PMN lysates. It also corresponds closely to the K_m of 2.8×10^{-6} M obtained for histaminase purified from human placenta [15].

Significant inhibition of histaminase activity was observed with each of the three H-2 antagonists at concentrations of 10^{-5} M and greater (Fig. 2). Burimamide was particularly potent, causing complete inhibition at 10^{-5} M. In contrast, diphenhydramine, an H-1 antagonist, possessed minimal inhibitory activity; pyrilamine, chlorpheniramine and cyclizine were similarly without effect. Of the H-1 antagonists examined, only promethazine produced significant inhibition (50 per cent at 10^{-4} M but none at 10^{-5} M). Thus, with the possible exception of the phenothiazine class, the inhibition was specific for the H-2 antagonists. The inhibition by phenothiazines is being pursued further. A similar pattern of inhibition was obtained for H-2 antagonists with histaminase prepared

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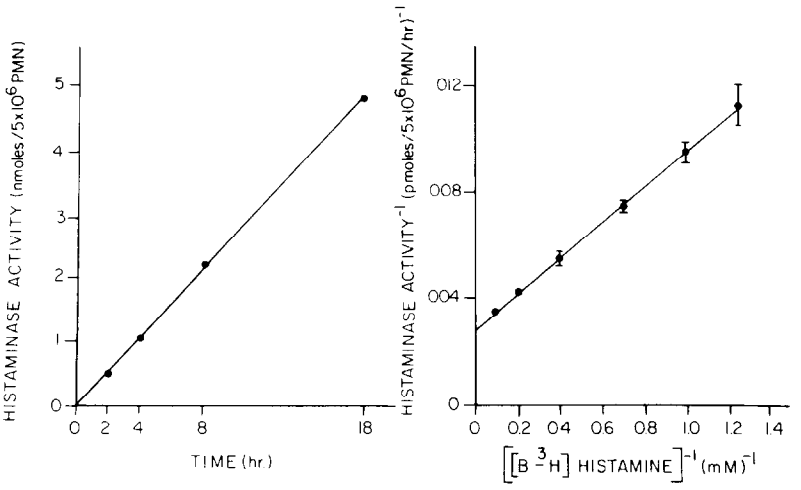

Fig. 1. Kinetics of PMN-derived histaminase activity. Left panel: PMN supernatant fraction was incubated with 4.5 μM [β-^{3}H] histamine (37 mCi/m-mole) for the times indicated. Background activity, determined by substituting TACM for PMN supernatant fraction, ranged from 83 cpm for 2 hr to 355 cpm for 18 hr. Aminoguanidine (10^{-5} M)-inhibited enzyme yielded the same background activity. A histaminase activity of 1 nmole/5 $\times$ 10^6 PMN was equivalent to 490 net cpm. The net mean activity for duplicate determinations at each time point is given. Right panel: PMN supernatant fraction was incubated with [β-^{3}H]histamine for 8 hr. Specific activity of the substrate at the two higher concentrations was 4.2 mCi/m-mole. The mean $\pm$ S. D. for triplicate determinations in a single experiment is shown, except where the S. D. was too small to depict. The line was fitted by least squares linear regression analysis.

from an additional donor. Double reciprocal plots of histaminase activity vs substrate concentration in the presence of the H-2 blockers indicated that each inhibited histaminase activity in a competitive fashion (Fig. 3). Inhibitory constants, K_I, of 2.3 $\times$ 10^{-7} M, 7.7 $\times$ 10^{-6} M and 2.7 $\times$ 10^{-5} M were calculated for burimamide, cimetidine and metiamide respectively.

The results indicate that human PMN-derived histaminase activity is inhibited by pharmacological concentrations of burimamide and cimetidine, and to a lesser extent metiamide. It has previously been reported that burimamide and cimetidine at concentrations up to 10^{-2} M had no effect on activity of diamine oxidase isolated from dog small intestine [16]. The reason for this discrepancy is not clear. Burimamide and metiamide, however, have been reported to inhibit histamine methyltransferase (HMT) activity [17, 18], in agreement with reports that H-1 antagonists inhibit HMT activity [19, 20]. Unlike the results presented here, inhibition of HMT activity required concentrations in the range of 10^{-3} M for each class of antagonist. The PMN-derived histaminase, therefore, appears significantly more sensitive to

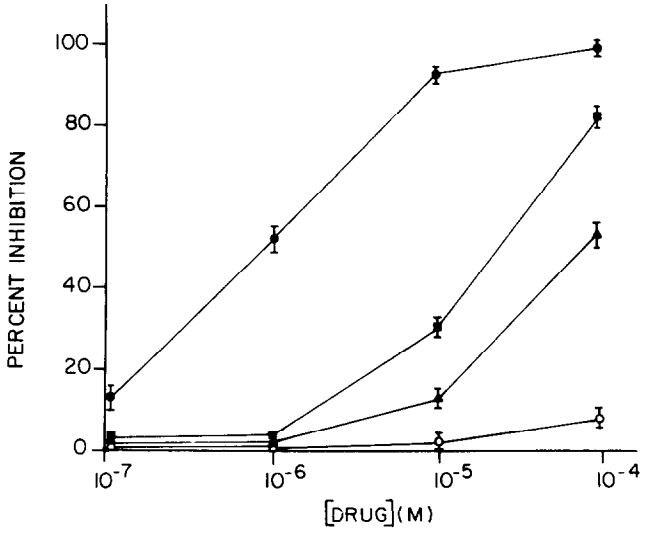

Fig. 2. Inhibition of histaminase activity by histamine antagonists. PMN supernatant fractions were incubated with 4.5 μM [β-^{3}H]histamine (37 mCi/m-mole) for 16 hr in presence of burimamide (●), cimetidine (■), metiamide (▲) or diphenhydramine (○). The mean inhibition and range for two experiments, each done in duplicate are shown. Control activity was unchanged by the addition of H-2 antagonists at 10^{-4} M to the assay prior to stopping the reaction.

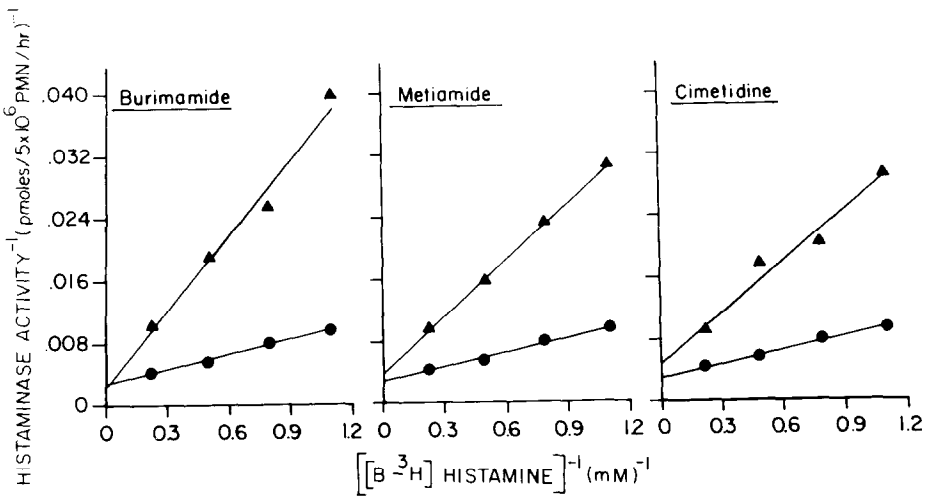

Fig. 3. Double reciprocal plots of histaminase activity vs substrate concentrations in the presence of H-2 antagonists. The PMN supernatant fractions were incubated as described in Fig. 2 for 8 hr with 8×10^{-5} M metiamide, 2×10^{-5} M cimetidine, or 10^{-6} M burimamide. Activity obtained in the absence (●) and presence (▲) of the antagonist is shown for each panel. Each point is the mean of duplicate determinations. Lines were fitted by least squares linear regression analysis.

inhibition by the H-2 blockers than does HMT. Inhibition of both HMT by H-1 antagonists [17] and histaminase by H-2 antagonists occurs by a competitive mechanism. Since H-1 antagonists retain the quaternary ammonium structure of histamine while H-2 antagonists possess the imidazole ring in common with histamine, it is implied that histamine is bound to HMT via the side chain and to histaminase via the imidazole ring. The finding that imidazole (10^{-4} M) caused a 50 per cent inhibition in activity is consistent with this interpretation; the product of the histaminase catalyzed reaction, imidazole acetic acid, produced no inhibition. The increased potency of burimamide as an inhibitor of histaminase may similarly by related to its structure. Whereas the imidazole rings of metiamide and cimetidine each have an attached methyl group, the imidazole ring of burimamide is identical to that of histamine.

The implications for inhibition *in vivo* of histaminase by H-2 antagonists are underscored by two recent reports of Yamamoto *et al.* [21, 22]. They observed that 10^{-5} M aminoguanidine completely abolished degradation of histamine by a homogenate of guinea pig skin. In addition, pretreatment of guinea pigs with aminoguanidine potentiated the 72-hr passive cutaneous anaphylaxis reaction, suggesting histaminase involvement in the metabolism of histamine released during anaphylactic reactions. Similarly, burimamide at 10^{-4} M completely inhibited degradation of histamine by the homogenate of guinea pig skin and retarded metabolism of histamine released during antigenic challenge of sensitized guinea pig skin [22].

Thus, H-2 antagonists may exert two opposing actions with respect to modulation of the immune system. While blocking the H-2 mediated effects of histamine on immune cells, H-2 antagonists may at the same time prolong the histamine action by inhibiting one of its primary metabolic pathways. It is important to note, however, that the potency of the H-2 antagonists differs for the two opposing actions. Whereas burimamide is the more potent inhibitor of histaminase activity, metiamide is more potent as an antagonist of the H-2 mediated effects of histamine on immune cells [11, 12]. This difference in sensitivity will still allow at least metiamide to act selectively only at the H-2 receptor. With a K_B for immune cells [11, 12] 10- to 100-fold less than its K_I, pharmacological concentrations of metiamide can be employed without influencing histaminase activity.

Clinical Immunology Division, Department of Medicine The Johns Hopkins University, School of Medicine, Baltimore, MD, U.S.A.

LARRY L. THOMAS*
BRUCE S. BOCHNER
LAWRENCE M. LICHTENSTEIN

* Recipient of National Institutes of Health Fellowship Award AI 05476.

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