with pure G_{M1} ganglioside and (ii) G_{M1} is the only ganglioside species that is labeled when intact synaptosomes are treated first with galactose oxidase and then with ^{3}H-labeled sodium borohydride (*8*).

The potential for specific localization by autoradiography or immunohistological methods is, of course, one of the major advantages of the immunological model. Another is that the absence of gross tissue damage may bring this model close in character to clinical epilepsies of nontraumatic origin. However, the most striking advantage arises from the capacity of immunological methods to provide molecular assignments to reaction sites and thus to indicate whether one or multiple antigenic sites are involved in disruption of synaptic pathways by formation of specific antigen-antibody complexes. As an experimental model of epilepsy, it is important to examine synaptic junctions in various brain regions for evidence of morphological change. Such an examination will have much greater significance when highly purified antibodies are administered rather than whole antiserum.

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9. Gangliosides were prepared from gray matter according to J. N. Kanfer [*Methods Enzymol.*, **14**, 660 (1969)] and used without alkaline hydrolysis. The preparation contained 27.2 percent sialic acid and 0.56 percent phosphorus. The four major ganglioside species, G_{M1}, G_{D1a}, G_{D1b}, and G_{T1} comprised more than 90 percent of this material. Antiserums were prepared in rabbits as described by T. A. Pascal, A. Saifer, J. Gitlin [*Proc. Soc. Exp. Biol. Med.* **121**, 739 (1966)].
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11. The three antiserums to gangliosides used in our study had antibody titers greater than 150 against pure G_{M1} ganglioside in a complement fixation test with 4 units of complement. Absorptions were carried out as follows. To a solution of 25 μg of pure G_{M1} and 50 μg of lecithin in 0.05 ml of ethanol and 0.45 ml of saline, 0.5 ml of antiserum was added. After incubation with stirring at 25°C for 2 hours, the solution was kept at 4°C overnight and then centrifuged at 24,000*g* for 1 hour at 4°C. The upper 0.95 ml of clear supernatant was used for testing. Unabsorbed antiserum was treated in the same way, but without addition of the ganglioside-lecithin mixture.
12. A 25-gage needle held rigidly by a stereotaxic manipulator was inserted to a depth of 1.5 mm below the exposed dura over the sensorimotor cortex. Histological examination showed that deepest penetration of tissue was 0.5 to 1.0 mm.
13. Brains were embedded in paraffin, coronal sections (10 μm) were taken from an area extending 4 mm anterior and 4 mm posterior to the locus of injection, and stained with hematoxylin and eosin.
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Histamine Inhibition of Neutrophil Lysosomal Enzyme Release: An H2 Histamine Receptor Response

Abstract. *Human polymorphonuclear leukocytes treated with cytochalasin B release the lysosomal enzyme beta glucuronidase during contact with serum-activated zymosan particles. Histamine increases intracellular cyclic adenosine monophosphate and inhibits release of this enzyme. The H2 antihistamine metiamide blocks the histamine inhibition of lysosomal enzyme release and the increase in the intracellular adenosine 3,5'-monophosphate of granulocytes. Chlorpheniramine, an H1 antihistamine, did not block the histamine inhibition of granulocyte lysosomal enzyme release.*

Aside from its well-known role as a mediator of anaphylaxis, the physiological importance of histamine remains largely undefined (*1*). Recent evidence suggests that histamine also has an important modulating effect on the function of a variety of cells involved in immunity and inflammation. For example, histamine will inhibit leukocyte chemotaxis (*2*); the release of lysosomal enzymes (*3*); antigen-induced IgE mediated release of histamine from peripheral leukocytes (*4*); the cytolytic activity of effector T lymphocytes in mice (*5*); and the release of macrophage migration inhibitory factor by sensitized lymphocytes (*6*). These last three effects are mediated by H2 histamine receptors. We report here that histamine inhibition of the release of a lysosomal enzyme, β-glucuronidase (E.C. 3.2.1.31), from human neutrophils in the presence of serum-activated zymosan is an H2 receptor response probably mediated through adenosine 3,5'-monophosphate (cyclic AMP).

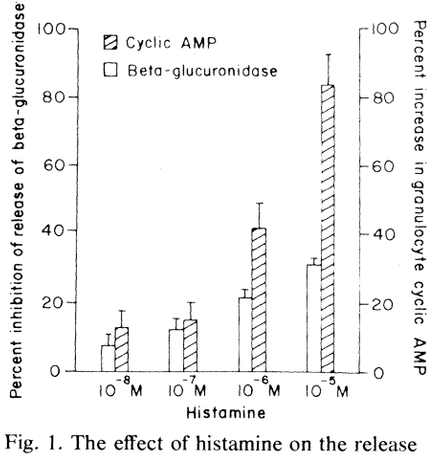

Fig. 1. The effect of histamine on the release of β-glucuronidase from neutrophils exposed to serum and zymosan and changes in the concentration of neutrophil cyclic AMP. Each result represents the mean ± standard error of five separate experiments. Human neutrophils (3×10^6 in 1 ml of HBSS) were incubated for 5 minutes with cytochalasin B (5 μg/ml, final concentration) at 37°C; they were then incubated for 15 minutes with histamine, and then for 30 minutes with zymosan and serum. Control incubations (no histamine) yielded glucuronidase value of 22.2 ± 0.7 (mean ± standard error) μg of phenolphthalein per 18 hours per 10^6 neutrophils which was 32.8 percent of the total enzyme activity. Beta glucuronidase release without serum and zymosan was 2.5 ± 0.5 μg of phenolphthalein per 18 hours per 10^6 neutrophils. Baseline cyclic AMP concentration was 1.25 ± 0.13 picomole per 10^6 neutrophils.

Ash and Schild (*7*) postulated that at least two types of receptors were involved in the histamine response. Responses of the H1 histamine receptor include vasodilatation and smooth muscle contraction which are blocked by antihistamines such as mepyramine, diphenhydramine, and chlorpheniramine. Gastric acid secretion, rat uterine muscle relaxation, and guinea pig atrium contraction follow histamine stimulation of receptors not blocked by the above antihistamines. It was possible to confirm the presence of H2 histamine receptors when the new antagonists, burimamide, metiamide, and cimetidine, were discovered which specifically block the second group of responses to histamine, but not the first (*8*).

Enzymes released from granulocyte

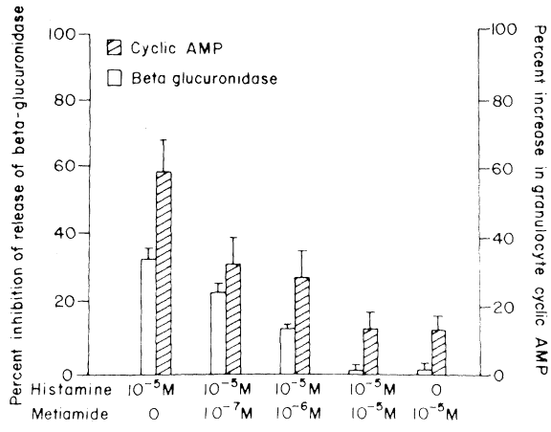

Fig. 2. Effect of the H2 antihistamine metiamide on the effect of histamine on neutrophil cyclic AMP and on β-glucuronidase release from human neutrophils exposed to serum and zymosan. Each result represents the mean ± standard error of four experiments. Human neutrophils (3×10^6 in 1 ml of HBSS) were incubated with cytochalasin B (5 μg/ml, final concentration) for 5 minutes at 37°C; metiamide was added immediately prior to 15 minutes of incubation with histamine. The cells were then incubated with serum and zymosan for 30 minutes at 37°C.

lysosomes are thought to play a major role in the inflammatory process (*9*). Suppressing the release of these enzymes is one possible mechanism by which anti-inflammatory agents such as salicylates (*10*) and hydrocortisone (*11*) exert therapeutic benefit. The secretion of endogenous chemical agents such as catecholamines may also play a role in regulating the inflammation (*3, 12*). Epinephrine and other catecholamines inhibit granulocyte lysosomal enzyme release (*13*) and this activity is thought to be mediated by cyclic AMP (*14*).

By means of Ficoll-Hypaque density-gradient separation, human polymorphonuclear leukocytes were isolated from anticoagulated blood (2.7 percent EDTA) (*15*). Isolated granulocytes were suspended in Hanks balanced salt solution (HBSS) in a concentration of 3×10^6 polymorphonuclear leukocytes per milliliter. Zymosan particles were boiled in saline (10 mg/ml), washed, and resuspended in HBSS (10 mg/ml). Replicate samples (3×10^6 polymorphonuclear leukocytes in 1 ml of HBSS, 98 percent polymorphonuclear leukocytes) were incubated in a shaking water bath for 5 minutes at 37°C with cytochalasin B (5 μg/ml, final concentration); 0.1 ml of fresh autologous serum and 0.1 ml of the prepared zymosan were then added to each sample (*16*) which was then incubated for 30 minutes at 37°C. The samples were centrifuged (750*g* for 10 minutes at 4°C) and the concentration of the lysosomal enzyme β-glucuronidase (*17*) and the cytoplasmic enzyme lactate dehydrogenase (E.C. 1.1.1.27) (*18*) were measured in the supernatant. Total enzyme activity was determined after the cells were lysed with Triton X-100 (0.2 percent).

The effect of histamine on the release of β-glucuronidase was determined by incubating the cells with freshly prepared histamine diphosphate for 15 minutes at 37°C prior to adding serum and zymosan. The release of β-glucuronidase in the presence of histamine was compared to that with zymosan and serum alone. In the experiments with antihistamines, these drugs were added immediately prior to the addition of histamine. All studies with drugs were performed in the presence of theophylline ($5 \times 10^{-4}M$) which itself did not alter either enzyme release or cellular concentrations of cyclic AMP.

Neutrophil cyclic AMP was extracted in ethanol, dried with nitrogen, and then measured by the saturation assay method (*19*).

Histamine inhibited the release of β-glucuronidase in a dose-dependent manner over the range of $10^{-8}M$ to $10^{-5}M$ (Fig. 1). An increase in cyclic AMP in the polymorphonuclear leukocytes paralleled the histamine inhibition of enzyme release. Maximum stimulation of adenylate cyclase (E.C. 4.6.1.1) was found with $10^{-5}M$ histamine. Cell viability was indicated by a less than 3 percent increase in supernatant lactate dehydrogenase in all experiments.

The H2 antihistamine metiamide blocked the effect of histamine on lysosomal enzyme release (Fig. 2). At equimolar strengths metiamide completely blocked histamine inhibition of enzyme release. The H1 antihistamine chlorpheniramine had no effect on this histamine response. Adenylate cyclase stimulation by histamine was similarly blocked by metiamide.

Our data indicate that neutrophils have H2 receptors and that stimulation of these receptors inhibits the release of enzymes from neutrophil lysosomes. The degree of inhibition of enzyme release by histamine is associated with increasing intracellular cyclic AMP, like the effect of epinephrine and other catecholamines.

Our observations may clarify the role of histamine in inflammation. Stimulation of H1 receptors produces an increase in capillary permeability which is part of the inflammatory response. Immune complex deposition and activation of the complement system generate the complement components C3a and C5a that are not only chemotactic for leukocytes but also intensify the inflammation by stimulating the release of lysosomal enzymes. These same complement components, C3a and C5a, release histamine from mast cells. Histamine may then in turn reduce the inflammatory response by stimulating H2 receptors on granulocytes, thereby inhibiting lysosomal enzyme release. Thus, histamine may have either an amplifying or an inhibiting effect on the inflammatory response depending upon the stage of the inflammation, upon the cell type involved, and upon whether H1 or H2 receptors are stimulated.

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