Receptors for Histamine Can Be Detected on the Surface of Selected Leukocytes

Abstract. *Histamine insolubilized by chemical linkage via a protein or polypeptide carrier to Sepharose beads cannot penetrate cells. Even so, the resultant histamine-coated Sepharose binds leukocytes selectively. Despite the low molecular weight of histamine, the binding is via preformed cell membrane receptors and can be specifically and competitively blocked or reversed by antihistamines.*

Specific membrane receptors have been described for a number of polypeptide hormones such as vasopressin, adrenocorticotropin, growth hormone, and insulin (*1*). The investigators first insolubilized the hormones so that they could not enter cells. Then they established the existence of membrane receptors and showed that it was sufficient for the insoluble substances to combine with receptor sites in order to produce intracellular effects (*2*). In parallel with these studies, investigations of the immune response have shown that small molecules can be recognized by receptors on a variety of immunocompetent cells (*3*). A number of such studies also employed a technique which made antigens or haptens unavailable to the intracellular environment by attaching them to beads and then allowing the critical chemical moiety to attach to the cell bearing a receptor (*4*).

We have previously shown that human leukocytes have specific and separate receptors for endogenous hormones, including beta-adrenergic catecholamines (epinephrine > norepinephrine), histamine, and prostaglandins ($PGE_1 > PGE_2 > PGF_{2\alpha}$) (*5*). All these agents stimulated production of cyclic adenosine monophosphate in leukocytes. The effects of the catecholamines were blocked by beta but not by alpha adrenergic blocking agents or by antihistamines; the effect of histamine was blocked by antihistamines but not by adrenergic blocking agents; and the effect of prostaglandin was not inhibited by either antihistamines or adrenergic blocking drugs. Each pharmacologic agonist stimulated leukocyte adenyl cyclase but presumably acted by first attaching to a specific pharmacologic receptor somewhere in or on the cell. Even though the amines and lipids in question have molecular weights of less than 400 (considerably less than the polypeptide endocrine hormones or immunoglobulins mentioned above), it was of interest to determine whether attachment of histamine to an insoluble carrier might bind cells in the same way that antigens bind to antibody-like receptors on lymphocytes. The object of this report is to demonstrate that there are specific binding sites for histamine on leukocytes. The receptors must recognize an extraordinarily small chemical determinant of the histamine but must be specific since the interaction with the insolubilized histamine is prevented by four antihistamines. The latter drugs have diverse chemical and pharmacologic properties but each is a competitive antagonist of histamine.

Blood from hematologically normal adult human volunteers was drawn into plastic syringes containing heparin (5 units per milliliter of blood). One volume of 3 percent dextran was added for each two volumes of blood, and the erythrocytes were allowed to sediment for 1 hour. Leukocytes in the upper layer were separated from plasma by centrifugation and washed once in 0.32*M* sucrose. The resulting preparation contained 15 to 20 leukocytes per platelet and 1 leukocyte per erythrocyte. Cells from animals were teased from various organs and suspended in Eagle's minimal essential media or phosphate-buffered saline (PBS) after straining through a stainless steel mesh (200 mesh). Different batches of agarose beads [0.3 ml of a 25 percent suspension of agarose/PBS (0.15*M* NaCl in 0.01*M* sodium phosphate, *p*H 7.1)] were incubated with 5×10^6 to 10×10^6 leukocytes from various sources for 15 minutes at 37°C. The Sepharose was attached to either (i) histamine, (ii) histamine or norepinephrine conjugates to rabbit serum albumin (RSA), bovine serum

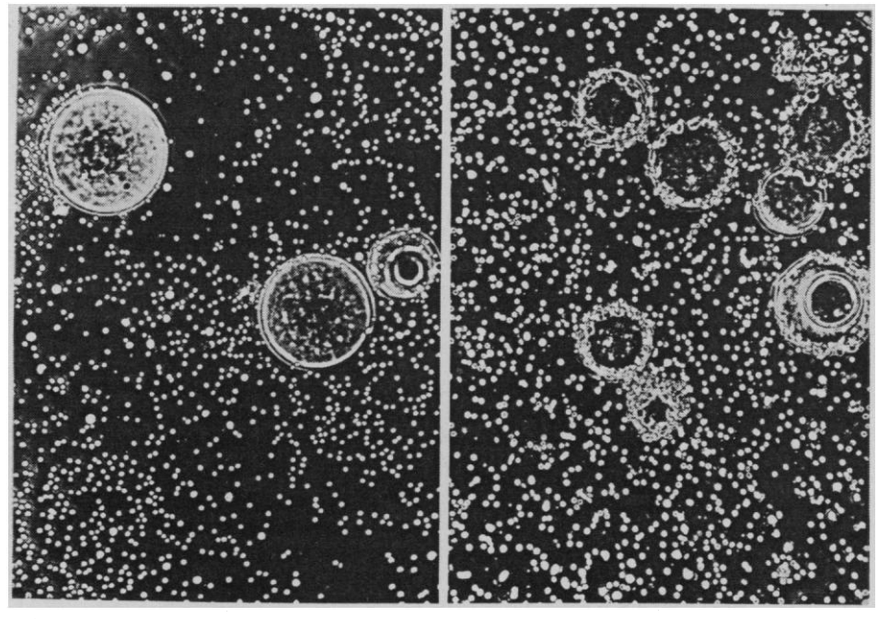

Fig. 1. Photograph (× 290) of mixed human leukocytes and erythrocytes (ratio of leukocytes to erythrocytes, 1 : 1) in the presence of RSA-Sepharose (left) and histamine-RSA-Sepharose (right). Note no attachment of cells to the control and selective attraction of leukocytes, at right. Erythrocytes can be recognized by their size and the open area in their centers. Agarose beads (Sepharose 4B, Pharmacia, Uppsala) were prepared after the method of Porath *et al.* (*6*). The beads were activated by cyanogen bromide (Eastman Chemicals). The washed, activated Sepharose was then interacted with one of three types of substances (in 0.1*M* $NaHCO_3$): (i) histamine dihydrochloride (Calbiochem), 5 mg per gram of wet Sepharose; (ii) histamine conjugated either to rabbit serum albumin (RSA, Mann), bovine serum albumin (BSA, Mann) or a random amino acid copolymer poly (DLAla^{95}, Tyr^{15}) via its amino function to the carbodiimide-activated (1-ethyl-3-3-dimethylaminopropyl carbodiimide HCl) (Ott Chemical, Muskegon, Mich.) carboxyl groups of the arm provided by the protein or polymer—2 mg per gram of wet Sepharose; or (iii) BSA, RSA, or the polymer—2 or 10 mg per gram of wet Sepharose. The histamine-protein conjugate contained 56 to 63 moles of histamine per mole of protein and 1 mole of histamine per mole of polymer. Norepinephrine-RSA-Sepharose (prepared like histamine-RSA-Sepharose) was also studied in the process of these studies. The activated Sepharose bound more than 95 percent of the conjugate used.

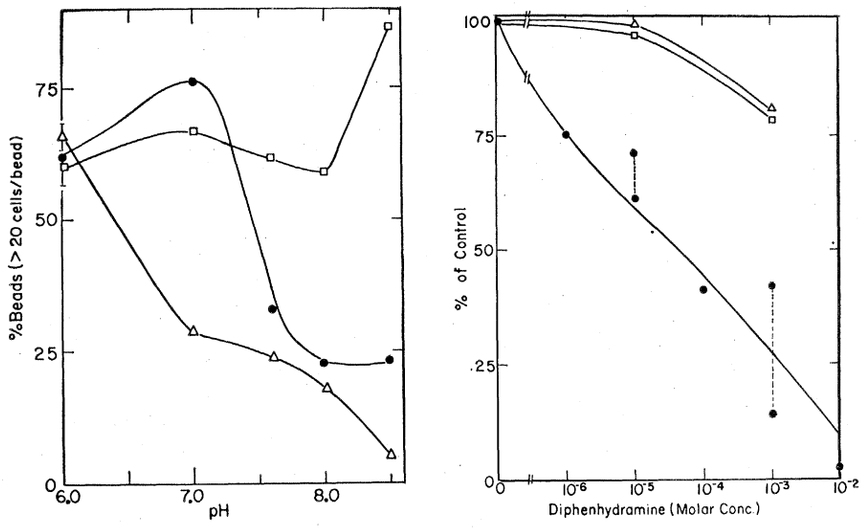

Fig. 2. Effects of *p*H changes (left) on binding of human leukocytes and of an antihistamine, diphenhydramine (right), in displacement of human leukocytes from histamine-RSA-Sepharose (●), polylysine-Sepharose (□), and polyethyleneimine-Sepharose (△). The differences in both sets of curves are also emphasized by the nonselectivity of binding by the polymers. Diphenhydramine could displace cells from the histamine-RSA-Sepharose only. Dotted lines joining solid circles represent the range in two separate experiments.

albumin (BSA), or a random amino acid copolymer, or (iii) to BSA, RSA, or the polymer by the method described by Porath *et al.* (*6*). After incubation, the cells and the beads were examined under the microscope and the beads were scored as binding if they held more than 20 cells, and as not binding if their cell content was less than 20 (Fig. 1).

Histamine-RSA-Sepharose bound a majority of human leukocytes (Fig. 1, right) but not platelets or erythrocytes. The differential count of unbound leukocytes was identical before and after incubation with beads, indicating the chance of binding of any cell was proportionate to its concentration. Regardless of the source of cells there was little or no binding to RSA-Sepharose, to activated Sepharose, to Sepharose alone, or to histamine attached directly to Sepharose (a univalent attractant). When beads were incubated with human leukocytes, more than 95 percent of the spheres were coated with cells. At the same ratio of beads to leukocytes, 40 to 50 percent of beads were coated with spleen cells from Balb/Bl mice and from Brown Norway rats, whether or not the animals were previously immunized with sheep red cells. Only 3 to 14 percent of beads were coated with leukocytes from the spleens of Lewis rats; 97 percent of beads were coated with peritoneal macrophages evoked by injection of thioglycolate into the peritoneal cavity of Balb/Bl mice; 12 to 15 percent of beads were coated with thymus cells from the Lewis or Brown Norway rat; 93 percent of beads were coated with cells from the lymph nodes of Brown Norway rats; but only 4 to 6 percent of beads were coated with cells from lymph nodes of the Lewis rat. Only 5 to 10 percent of beads were coated with mouse Balb/C myeloma cells. Thus considerable selectivity of binding was demonstrated among species and among leukocytes derived from different tissues of the same animal.

The characteristics and specificity of binding were studied by using human peripheral leukocytes. Binding of cells was maximal within 15 minutes, whether the incubation was done at 4° or 37°C. Cells held in PBS in the cold for 2 days could bind as well as freshly prepared cells. The binding was *p*H dependent (Fig. 2, left), but maximal binding did not correspond to the *pK* value of histamine ionization. Indeed, when other chemicals with a broad spectrum of *pK* values (piperidine, *pK* 11.2; pyridine, *pK* 5.2; and histidine with imidazole, *pK* 6.2) were added at 10^{-3} *M* to the mixture of cells and histamine-RSA-Sepharose, they did not displace cells from or prevent binding to more than 18 percent of the beads. When Sepharose was linked to polylysine or to polyethyleneimine (both basic polymers), each bound all types of cells (erythrocytes as well as leukocytes), but these were not displaced by the antihistamine, diphenhydramine, which was capable of releasing leukocytes from histamine-RSA-Sepharose (Fig. 2, right). Moreover, the *p*H dependence of the binding of cells to Sepharose coated with the basic polymers was very different from the pattern characteristic of histamine-RSA-Sepharose (Fig. 2, left). In addition, Sepharose to which histamine was conjugated via the synthetic copolymer or via BSA, bound cells in the same way as histamine-RSA-Sepharose. Such results indicate that the binding of cells is not simply due to ionic attraction and is substantially determined by the histamine itself and not its "carrier."

The binding of cells was specific for histamine, as illustrated by three additional experiments using human leukocytes:

1) Preincubation of cells with four antihistamines (4×10^{-3} *M*) prevented binding of the cells to histamine-RSA-Sepharose. Diphenhydramine and tripelennamine were equipotent (50 to 80 percent inhibition) and were followed by antazoline (45 percent inhibition) and pyrilamine (20 percent inhibition). Diphenhydramine, tripelennamine, and antazoline were also able to displace cells from the histamine-RSA-Sepharose beads and to prevent cell clumping by 0.25 mg of histamine-RSA per 5×10^6 cells per milliliter.

2) Histamine at 10^{-2} *M* produced 27 percent inhibition of binding. It was not surprising that high concentrations of monovalent histamine were relatively inefficient in displacing cells from or preventing binding to Sepharose linked to polyvalent RSA containing numerous histamine residues. The analogy in immunology is the inefficiency of displacement by means of monovalent haptens of specific cells from haptenated beads (*4*). The ability of histamine to prevent binding was limited but consistent, whereas comparable concentrations of norephinephrine, epinephrine, or prostaglandin E_2 had no inhibitory effect. Logically, if there are separate pharmacologic receptors for each of these small endogenous substances, the agonists would not be expected to share a common chemical binding site. Therefore they would not be expected to compete with each other for, or to displace each other from, a binding site. Thus these experiments are in accord with our previous demonstration (*5*) that the leukocyte histamine receptor (detected by measurement of cyclic adenosine monophosphate pro-

duction) is separate from the receptors specific for prostaglandins or beta-adrenergic catecholamines.

3) Norepinephrine-RSA-Sepharose did not bind leukocytes, an observation which correlates with its relatively low potency in producing cell surface effects. We are presently testing the ability of histamine-RSA-Sepharose to stimulate leukocyte adenyl cyclase. Such experiments should help in determining whether the free portions of the histamine on Sepharose are sufficient for both biologic effect and binding.

We have demonstrated in this study that methods used to define the relationships of cell surfaces to large proteins or haptens are applicable to small pharmacologically active agents. The insolubilized histamine can be used to separate morphologically similar cells to test whether they are biologically different. The histamine receptors of circulating leukocytes are not confined to the small number of cells which contain intracellular histamine [the basophils (*7*)], thus setting the stage for a chemical basis of cell cooperation or interaction. Furthermore, we may now be able to distinguish between intra- and extracellular effects of amines by studying the effects of free hormone on cells which do not contain receptors to histamine. Finally, the possible significance of multiple hormone receptors on the same cells and the relationship of cell function to the presence of a receptor might also be defined by application of our techniques to problems in inflammation and immunology.

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Amphotericin B Potentiation of Rifampicin as an Antifungal Agent against the Yeast Phase of Histoplasma capsulatum

Abstract. *Rifampicin, at high concentrations, inhibited growth and RNA synthesis in the yeast phase of* Histoplasma capsulatum. *These effects were potentiated by low concentrations of amphotericin B. The combination of the two agents was fungicidal, whereas each alone, at much higher concentrations, was only fungistatic.*

The dimorphic fungus *Histoplasma capsulatum* is an important human pathogen which is worldwide in distribution and endemic in the mid and southern central United States. The polyene amphotericin B is the only effective therapeutic agent for the disseminated form of this infection; but it is only fungistatic, requires long-term therapy, and carries with it a high degree of toxicity (*1*). This report shows that rifampicin alone at high concentrations inhibited RNA synthesis in *H. capsulatum* and decreased the viability of the yeast-like phase. The effect of rifampicin was enhanced by amphotericin B, and the interaction of the two agents against *H. capsulatum* could be characterized as synergistic.

In tissue, *H. capsulatum* is found in

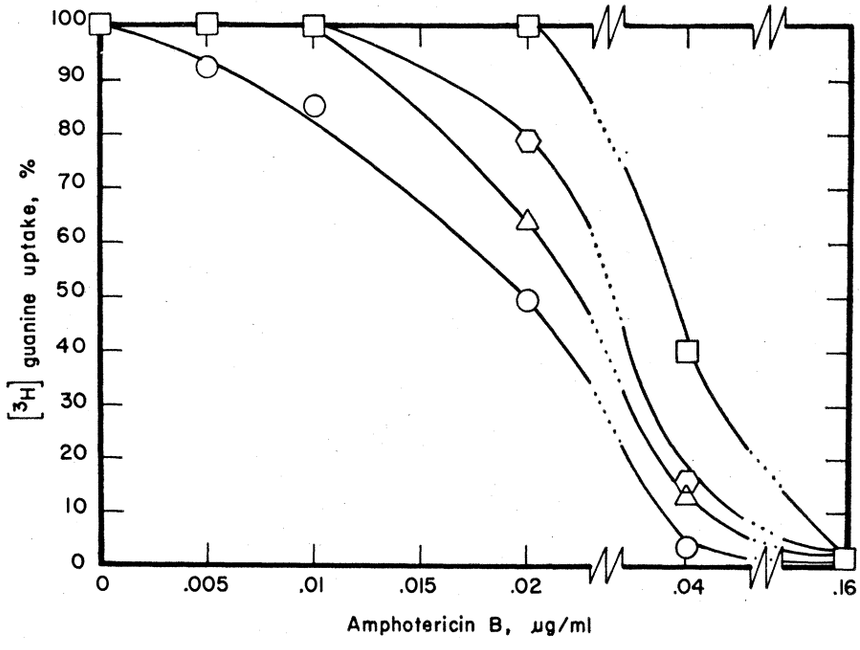

Fig. 1. Dose response to amphotericin B (Squibb) of [^{3}H]guanine incorporation in the presence or absence of rifampicin. Ten-milliliter volumes of a stock suspension of the yeast phase of *H. capsulatum* (Downs) (2×10^5 to 4×10^5 cell/ml) were dispensed into 50-ml erlenmeyer flasks with the appropriate agents and 0.5 μc of [^{3}H]guanine (specific activity, 13 c/mmole) per milliliter. Twenty-four hours later a portion of the culture was removed and precipitated with an equal volume of 10 percent trichloroacetic acid and filtered, and incorporation was determined. Duplicate samples were also removed and plated in triplicate to determine viability by colony counts. Symbols: □, no rifampicin; ○, 5 μg of rifampicin per milliliter; △, 10 μg of rifampicin per milliliter; ○, 20 μg of rifampicin per milliliter.