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CHARACTERIZATION OF HISTAMINE H-1 RECEPTORS ON HUMAN PERIPHERAL LUNG

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Abstract—Histamine H-1 receptors in human peripheral lung were characterized by radioligand and biochemical assays employing binding of the H-1 receptor antagonist [^{3}H]pyrilamine to plasma membrane preparations. Simultaneous computerized analyses of the data from fourteen separate equilibrium-binding assays indicated the presence of three distinct classes of binding sites with K_d values of 81 ± 35 pM, 7 ± 3 μM, and 320 ± 167 μM and binding capacities of 23 ± 3 pmoles, 10 ± 5 nmoles, and 297 ± 119 nmoles/mg protein respectively. Dissociation kinetics of [^{3}H]pyrilamine binding also supported the presence of three binding sites or states. Further, competition binding curves for histamine receptor agonists and antagonists also indicated the presence of multiple binding sites for the H-1 receptor. The effect of exogenous stimulation of histamine H-1 receptors on human cyclic nucleotides was also examined. Both histamine and the H-1 agonist 2-methyl histamine caused dose-related increases in the cyclic guanosine monophosphate (GMP) content of human lung. The effects of 2-methyl histamine were selective for cyclic GMP. The histamine-induced increase in cyclic GMP peaked within 1.0 min and was effectively prevented by the H-1 antagonist pyrilamine. Thus, human lung possesses a large number of H-1 receptors which exhibit three binding states and produce cyclic GMP, but not cyclic adenosine monophosphate (AMP), when stimulated.

The pathophysiologic events leading to allergic bronchial asthma involve an interaction between inhaled allergens and immunoglobulin E (IgE)§ molecules fixed to the surface of mast cells. In addition, anaphylatoxins [1, 2], radio-contrast media [3], many drugs (e.g. Refs 4–7), physical stimulants [8], and neurohormones [9–11] may all cause mast cell degranulation under appropriate conditions. In the human lung where mast cell mediators play an important role in the pathogenesis of asthma, mast cells are present in concentrations of 1–7 million cells/g of lung tissue [12] and may comprise 2% of alveolar tissue [13]. The resultant secretion of granules from the mast cell releases or generates the mediators of anaphylaxis [12, 14]. Of the mast cell-derived mediators, histamine is released immediately and may achieve local concentrations of >10 μM.

Two cellular receptors for histamine, termed H-1 and H-2 receptors, mediate the biologic effects of histamine [15]. Stimulation of H-1 receptors in the respiratory tract results in a number of physiologic responses including mucosal edema [16], the constriction of central and peripheral airways [17–20], increased pulmonary vascular resistance [21], production of cyclic guanosine monophosphate (GMP) [22], production of prostaglandins from both peripheral lung tissue and airways [22, 23] and irritant receptor stimulation [24]. Thus, it was of interest to characterize these human peripheral lung H-1 receptors by radioligand binding assays and to further investigate the cyclic nucleotide responses of human lung to exogenous stimulation of these receptors. Human lung was found to have a large number of H-1 binding sites that exhibited three binding states by both equilibrium and kinetic radioligand binding studies. Specific stimulation of these H-1 sites resulted in a rapid increase in the cyclic GMP, but not the cyclic adenosine monophosphate (AMP), content in lung tissue.

MATERIALS AND METHODS

Reagents. Cyclic AMP, cyclic GMP, cyclic AMP-dependent protein kinase, diphenhydramine, histamine diphosphate, pyrilamine, D,L-histidine, dopamine (3-hydroxytryptamine, D,L-isoproterenol, D,L-norepinephrine, phenol-red, Trizma HCl, and phenylmethylsulfonyl fluoride (PMSF) were purchased from the Sigma Chemical Co., St. Louis, MO; sucrose was purchased from the Fisher Scientific Co., Fair Lawn, NJ; $MgCl_2$ was from MCB Manufacturing Chemists, Inc., Cincinnati, OH; bovine serum albumin (BSA) was from Miles Laboratories, Inc., Elk-

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§ Abbreviations include: AMP, adenosine monophosphate; BSA, bovine serum albumin; *C*, proportionality (scaling) factors; ED_{50}, the dose of unlabeled ligand yielding 50% displacement of the labeled ligand [^{3}H]pyrilamine; GMP, guanosine monophosphate; IgE, immunoglobulin E; *K*, binding affinity; K_d, dissociation constant ($1/K$); *N*, nonspecific binding as calculated from the computer program LIGAND; PMSF, phenylmethylsulfonyl fluoride; *R*, binding capacity; and RMS, root-mean-square.

hart, IN; Tris and cyclic nucleotide phosphodiesterase beef heart were from Boehringer Mannheim, Indianapolis, IN; [^{3}H]cyclic AMP (37.7 Ci/mmole), [8-^{14}C]cyclic AMP (45 mCi/mmole), and [^{3}H]pyrilamine (27.3 Ci/mmole) were purchased from the New England Nuclear Corp., Boston, MA; D- and L-chlorpheniramine were gifts from the Schering Corp., Bloomfield, NH; cimetidine, metiamide, 2-methyl histamine and 2-(2(pyridyl) ethylamine were gifts from Smith Kline & French Laboratories, Philadelphia, PA; pyrilamine maleate was a gift from Merck, Sharp & Dohme Research Laboratory, West Point, PA; monospecific rabbit anti-cyclic GMP antisera and [^{125}I]2′*O*-succinyl-cyclic-GMP-tyrosine methyl ester were purchased from Collaborative Research, Inc., Waltham, MA; lyophylized rabbit serum was from ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, OH; goat anti-rabbit gammaglobulin was from Cappel Laboratories, Inc., Cochranville, PA; AG 1-X8, 200–400 mesh, formate was from Bio-Rad Laboratories, Richmond, CA; and Azocoll was from the Calbiochem-Behring Corp., La Jolla, CA.

Preparation of human lung tissue. Human lung tissue was obtained at the time of resection, which was generally for cancer or bronchiectasis. Macroscopically normal areas of peripheral lung tissue were dissected free of pleura, large bronchi (greater than 3–5 mm), and large blood vessels and washed extensively in Tris–HCl buffer, pH 7.4. The tissue was further dissected into 200-mg replicates (wet weight) for experiments involving the determination of cyclic nucleotide concentrations. Tissue for the radioligand binding assays was frozen at −70° until use (within 3 months).

Membrane preparation for radioligand binding assays. Frozen lung tissue was minced and placed in ice-cold 10 mM Tris (pH 7.4) containing 0.25 M sucrose, 0.5 mM PMSF, and 0.5% BSA (10 ml/g tissue). The tissue was homogenized using a Polytron PCU-2 homogenizer at 22,000 rpm for 2 min. The homogenate was filtered through double-layered gauze cloth and then rehomogenized for 30 sec at 22,000 rpm. The homogenate was then centrifuged twice at 900 *g* for 10 min at 4° to remove unbroken cells, cellular debris and nuclei. The protein content of the resulting supernatant fraction was adjusted to 2–10 mg/ml with the buffer used for the radioligand binding assays. Protein concentration was determined by the method of Lowry *et al.* [25]. These plasma membrane preparations were found to have a significant amount of 5′-nucleotidase activity (plasma membrane marker). Further, initial binding studies comparing the binding of [^{3}H]pyrilamine to both the crude preparation used for these studies and a purified plasma membrane fraction revealed that both fractions had similar dissociation constants [26]. The plasma membrane preparations were also assayed for protease activity by incubating 100-μl aliquots at 37° for 15 min with 1 ml of Azocoll solution containing 5 mg of Azocoll/ml of PBS, pH 7.0. The reaction was terminated by centrifugation at 400 *g* for 3 min. Protease activity was determined by following the absorbency at 520 nm of the supernatant fractions, which contain the soluble products generated during Azocoll degradation. These results were then compared to standard curves developed with specific proteases. By the Azocoll technique, no protease activity could be detected in the plasma membrane samples.

Radioligand binding assays. Radioligand binding assays were done as described previously [27]. Aliquots (100 μl) of the membrane preparations suspended in buffer containing 0.125 M Tris and 0.025 M $MgCl_2$ were used in the binding assays in a final volume of 160 μl. The assay mixtures contained either increasing concentrations of radioligand ([^{3}H] pyrilamine) or a fixed concentration of radioligand and different concentrations of various agonists and antagonists. In the kinetic and drug competition studies, the lung membranes were incubated for various intervals of time with 20 μl of [^{3}H]pyrilamine, 10 or 75 nM, at 22°. In the equilibrium studies, the lung membranes were incubated for 30 min with [^{3}H] pyrilamine at 22° with and without 40 μl of 100 μM unlabeled pyrilamine. Incubations were terminated by adding 4 ml of ice-cold incubation buffer followed by rapid vacuum filtration of the samples through diphenhydramine-presoaked Whatman GF/C glass filters. The filters were washed immediately thereafter with 24 ml of ice-cold buffer, dried, and then assayed in a liquid scintillation system using a Beckman LS-9000 counter. All samples were run in duplicate to quadruplicate, and replicates differed from each other by less than 10%. Initial binding experiments done at 4°, 22° and 37° demonstrated no significant difference in binding at the three different temperatures. The assays were done at 22° as a matter of convenience.

Preparation of human lung tissue for the determination of cyclic nucleotide concentrations. The concentrations of cyclic AMP and cyclic GMP in human lung tissue were determined as previously described [22]. Lung fragments for the cyclic nucleotide assays were incubated with a histamine receptor agonist, an antagonist, or both for an appropriate time and transferred to 10% perchloric acid at 0–4°. To the fragments in the 10% perchloric acid, 3000 cpm of [^{14}C]cyclic AMP (0.1 pmole) and 3000 cpm of [^{3}H]-cyclic GMP (0.333 pmole) were added to permit quantification of recovery. The fragments were homogenized for 30 sec at 4000 rpm and centrifuged at 2000 *g* for 15 min. The precipitates were retained and digested in 2 ml of 0.1 N NaOH for 48–72 hr for protein determination [25], and the supernatant fractions were neutralized with 5 N KOH using phenol-red as a pH indicator. After centrifugation at 2000 *g* for 15 min, the supernatant fraction was applied to 1.5 × 0.7 cm columns of AG 1-X8, equilibrated in 0.1 N formic acid. The columns were washed sequentially with 10 ml of distilled water and 10 ml of 0.1 N formic acid. The cyclic AMP was eluted with 10 ml of 1 N formic acid, and the cyclic GMP was eluted with 15 ml of 4 N formic acid. The eluates were lyophilized and were then resuspended in acetate buffer (1.0 ml, 50 mM, pH 4.0, for cyclic AMP; or 0.5 ml, 50 mM, pH 6.2, for cyclic GMP). The cyclic AMP concentration was determined with the protein-binding assay [28] and that of cyclic GMP by radioimmunoassay [29].

The values of cyclic nucleotide concentrations reported herein have been corrected on the basis of

the recovery of 0.1 pmole of [^{14}C]cyclic AMP and 0.033 pmole of [^{3}H]cyclic GMP in each sample with the actual recoveries ranging from 70 to 85% for cyclic AMP and from 55 to 80% for cyclic GMP. The values for cyclic nucleotide concentrations are reported as fmoles or pmoles per mg of protein. Incubation of representative samples with cyclic 3′,5′-nucleotide phosphodiesterase before the assay produced more than 95% hydrolysis of both cyclic AMP and cyclic GMP in all cases.

Statistical significance was determined by the paired *t*-test. Variations of experimental samples from control samples with $P < 0.05$ were considered significant. The "n" in each result indicates the number of individual experiments pooled to generate the data. Each results is presented as the mean ± S.E.M.

Modeling and statistical evaluation of receptor binding. The binding data were analyzed using a weighted, nonlinear, least-squares curve fitting as provided in the computer program LIGAND [30]. The binding curves were first re-expressed in terms of bound pyrilamine concentration versus total concentration added, considering both the labeled (hot) and unlabeled (cold) ligand. Contrary to common custom for binding studies, nonspecific binding (N) was not measured for each individual concentration by use of a 100-fold excess cold ligand concentration. Rather, N was modeled directly as an extra, very low affinity, nonsaturable class of receptors [30]. This extended model was then fit to the "raw" (specific + nonspecific) binding data. This modified analysis permits the detection of low affinity sites because a wider range of ligand concentration is available. The parameter N is a measure of the presence of such nonsaturable binding and is the ratio of nonspecifically bound to free ligand.

Equilibrium binding models with one, two, or three classes of specific binding sites were fitted to the data, and the best-fitting model was chosen on the basis of an "extra sum-of-squares" F-test criterion [30]. That is, the addition of a new class of binding sites into the model had to result in a better fit to the data that was statistically significant ($P < 0.05$) before the new class was considered part of the model. Finally, values for the binding affinities (K), binding capacities (R), and N for the chosen model were estimated, along with their percent coefficient of variation, i.e. 100 × S.E./parameter value. The predicted curve was then plotted superimposed on the data, and the goodness-of-fit was evaluated using a "number-of-runs" test on the residuals and by evaluation of the root-mean-square (RMS) residual error.

When experiments involved different lung membrane preparations, some variability in the values for R was observed, even after the binding data were normalized for protein concentration. To reduce the effect of this variability, a set of proportionality (scaling) factors (C) was estimated for each series of experiments. These parameters essentially allow for proportional fluctuations in the R and N values from experiment to experiment, but require that the K values remain constant. By convention, the proportionality factor for the first experiment in a set (C_1) is set to 1. For example, if $C_2 = 2.05$, we would then understand that the second experiment had approximately twice the concentration of binding sites as the first.

During the computer analysis, R is calculated in molar concentration based in the incubation medium. For presentation, however, the R values were divided by the average protein concentration (mg protein/liter) so that comparable units for R (moles/mg protein) were obtained. The reported value of R is thus the average value obtained within a given set of experiments. Significant between-experiment variability can be effectively handled via this analytic method, as previously demonstrated [30].

The dissociation data were analyzed by fitting a multiexponential equation:

$$y = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} + D$$

using the MLAB modeling system on the DEC-system 10 computer at the NIH Division of Computer Resources and Technology, and by use of program EXPFIT, in BASIC for the DEC-10 (developed by V. G. Guardabasso, P. J. Munson and D. Rodbard; available on request).

RESULTS

Equilibrium binding studies with [^{3}H]*pyrilamine.* To analyze the pyrilamine binding to human lung membranes over a wide range of pyrilamine concentrations, it was necessary to pool data from multiple experiments and use normative correction factors that allow for proportional fluctuations in saturable and nonsaturable binding with constant K values. Equilibrium binding models with one, two, and three classes of specific binding sites were then fitted to the pooled data using the computer program termed LIGAND [30]. The optimal binding parameters for each of the models are listed in Table 1. With an F ratio test, the reduction in the RMS for the three-site model as compared to the one- and two-site models was highly significant ($P < 0.001$), indicating that the three-site model represent the best fit for the data, Introduction of an additional (fourth) class of sites, or of positive or negative cooperativity did not result in a statistically significant improvement in the goodness of fit. The computer-fitted curve for the three-site model generated by the analysis of the data from fourteen separate equilibrium binding assays is shown in Fig. 1. This curve illustrates the wide range of pyrilamine concentrations required to obtain accurate parameter estimates for the chosen model having three binding sites with affinities spanning several orders of magnitude.

Dissociation kinetics of [^{3}H]*pyrilamine binding to human lung.* The plots of four experiments (indicated by different symbols) examining the time course for dissociation of [^{3}H]pyrilamine binding to human lung plasma membranes is shown in Fig. 2. All four curves have the same nonlinear shape and confirm the multiplicity of binding states for the H-1 receptor. The data from all four experiments were most compatible with three distinct dissociation rate constants. The calculated rate constants for the four experiments were the same, and the $T_{1/2}$ values for dissociation (the time for one-half the radioligand concentration

Table 1. Binding capacities (R) and dissociation constants (K_d) for [^{3}H]pyrilamine binding to human peripheral lung

	K_{d1} (pM)	R_1 (pmoles/mg protein)	K_{d2} (μM)	R_2 (nmoles/mg protein)	K_{d3} (μM)	R_3 (nmoles/mg protein)	N ($\times 10^{-4}$)	RMS (%)	df*
One-site model	333 ± 147	50 ± 7					17 ± 0.5	36	244
Two-site model	119 ± 49	27 ± 3	66 ± 9	104 ± 14			7 ± 0.4	22	242
Three-site model	81 ± 35	23 ± 3	7 ± 3	10 ± 5	320 ± 167	297 ± 119	8 ± 0.7	18†	240

* Degrees of freedom.
† $P < 0.001$ by F ratio test compared to both the one-site and two-site models.

to be displaced from a binding site) were 153 min for the slow component, 2.5 min for the intermediate component, and <0.5 min for the rapid component. Due to the limitations of the technique used to separate the bound from the free ligand (membrane filtration), the $T_{1/2}$ of dissociation for the rapid component could only be approximated. Thus, by both kinetic and equilibrium binding studies, the human lung H-1 receptor appears to consist of three classes of binding states or sites.

Specificity (drug competition) studies. To measure the binding affinity of different drugs relative to the affinity of [^{3}H]pyrilamine for the H-1 sites, ED_{50} values were calculated from competition binding curves. The ED_{50} value is the dose of unlabeled ligand yielding 50% displacement of the labeled ligand [^{3}H] pyrilamine and was calculated by fitting multiple simultaneous logistic curves with the computer program ALLFIT [31]. The curves resulting from the competition or displacement plots consistently had slopes of less than one, suggesting competition at more than one class of binding sites. Figure 3 is an example of such curves, and it shows the displacement of [^{3}H]pyrilamine binding by another H-1 antagonist, diphenhydramine. Despite the use of different [^{3}H]pyrilamine concentrations (10 and 100 nM), both curves give the same ED_{50} value of approximately 44 μM, illustrating the reproducibility of the ED_{50} determinations. The rank order of potency for displacement of [^{3}H]pyrilamine binding from the H-1 binding site is the H-1 antagonists > H-1 agonists > histamine > H-2 antagonists (Table 2). Drugs not specific for histamine receptors had virtually no effect. Thus, by competition binding studies, the H-1 binding site exhibits the expected rank order of potency for an H-1 receptor.

Effects of histamine upon lung cyclic nucleotides. The time course of the cyclic GMP response to 10 μM

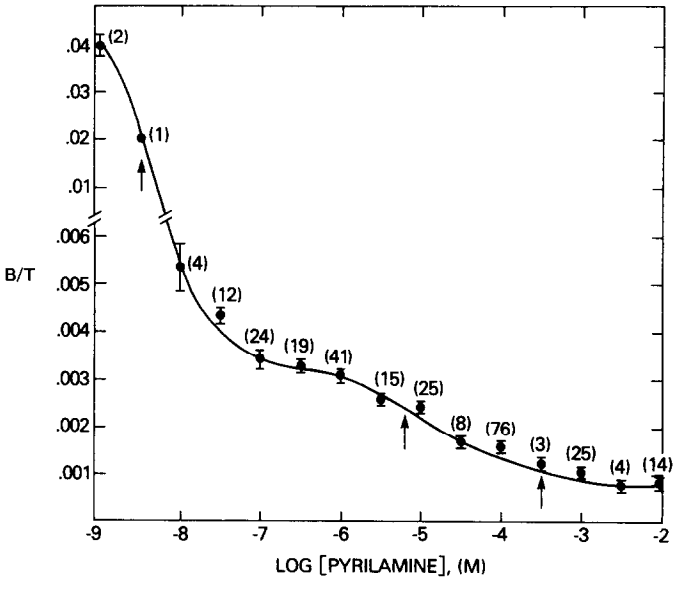

Fig. 1. Displacement by unlabeled pyrilamine of [^{3}H]pyrilamine binding from human peripheral lung membranes. This computer drawn curve plots the amount of pyrilamine bound over the total added (B/T) versus the log of total pyrilamine concentration. The points represent the mean ± S.E.M. B/T values for the corresponding pyrilamine concentrations. The number of observations at each point is indicated in parentheses. The curve shows a stepped appearance indicative of three distinct classes of binding sites labeled by the arrows.

Table 2. ED_{50} Values for competition of [^{3}H]pyrilamine binding from human lung plasma membranes

Agent	Action	ED_{50} [μM]
Pyrilamine	H-1 antagonist	8.8 ± 1.6
D-Chlorpheniramine	H-1 antagonist	21.5 ± 4.3
L-Chlorpheniramine	H-1 antagonist	40.3 ± 9.3
Diphenhydramine	H-1 antagonist	43.8 ± 14.3
2-(2-Pyridyl)ethylamine	H-1 agonist	241.2 ± 159.9
Histamine	H-1, H-2 agonist	3244.2 ± 243.1
Metiamide	H-2 antagonist	3550.6 ± 1242
Cimetidine	H-2 antagonist	3793.2 ± 613.5
D,L-Histidine		>10,000
Dopamine		>10,000
Isoproterenol	β Agonist	>10,000

histamine is shown in Fig. 4. Histamine induced a rapid rise in cyclic GMP content, which peaked within 1 min and returned toward baseline thereafter. The peak level of cyclic GMP was 6.9 times that of control. Therefore, a dose response of histamine (0.01 to 50 μM) was examined 1 min after stimulation. Histamine at these concentrations resulted in a dose-related increase in cyclic GMP levels (Fig. 5).

The effect of pyrilamine on cyclic GMP generation by added histamine was studied to determine whether the H-1 antagonist might have a specific inhibitory capacity (Table 3). Pyrilamine itself had no effect on the cyclic GMP content, but it did effectively abrograte the expected histamine-induced rise, implying that H-1 receptor stimulation was responsible for the cyclic GMP production. To further study the effect of H-1 receptor stimulation on cyclic nucleotide content, the capacity of the H-1 agonist 2-methyl histamine to simultaneously induce changes in cyclic GMP and cyclic AMP was examined (Fig. 6). Although 2-methyl histamine generated a dose-related increase in cyclic GMP content, this H-1 specific agonist did not cause a statistically significant change in cyclic AMP.

DISCUSSION

Analysis of H-1 binding sites in human lung initially employed equilibrium binding experiments using nanomolar concentrations of [^{3}H]pyrilamine because of a preliminary report suggesting that the K_d for [^{3}H]pyrilamine binding to human lung was

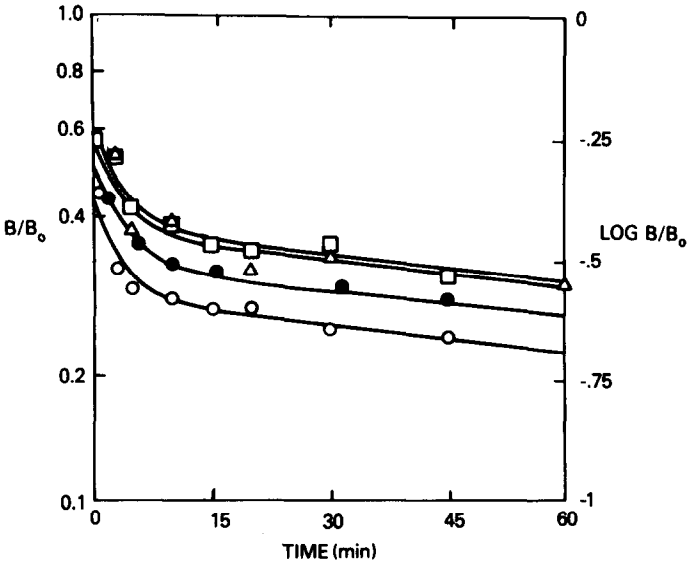

Fig. 2. Time course of dissociation of [^{3}H]pyrilamine binding to human lung plasma membranes. Lung membranes (1 mg protein) were incubated at 22° for 30 min with [^{3}H]pyrilamine (10 nM) followed by the addition at the 0 time point of an excess of unlabeled pyrilamine (10 mM). The log of the ratio of [^{3}H]pyrilamine bound at each time point (B) to that initially bound (B_0) is plotted as a function of time for four separate experiments (indicated by different symbols). Dissociation of binding at $t < 0.5$ min could not be accurately measured and is thus not clearly shown on the curves. Three rate constants were estimated from the data shown on these plots (see text).

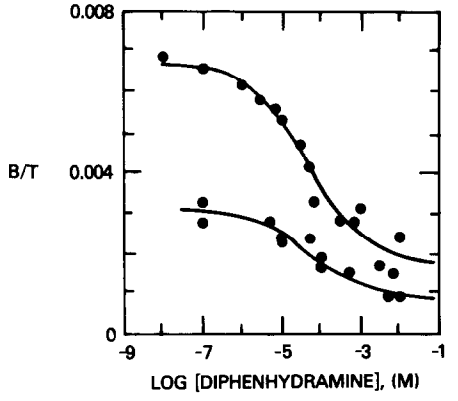

Fig. 3. Displacement of [^{3}H]pyrilamine from human lung membranes by unlabeled diphenhydramine. The curve shows the amount of [^{3}H]pyrilamine bound over the total (B/T) as a function of the amount of unlabeled diphenhydramine added. The upper and lower curves were generated from experiments using 10 and 100 nM [^{3}H]pyrilamine, respectively, and result in the same ED_{50} value of 44 μM. Both curves show a stepped appearance with slopes of 0.58, suggestive of multiple classes of binding sites.

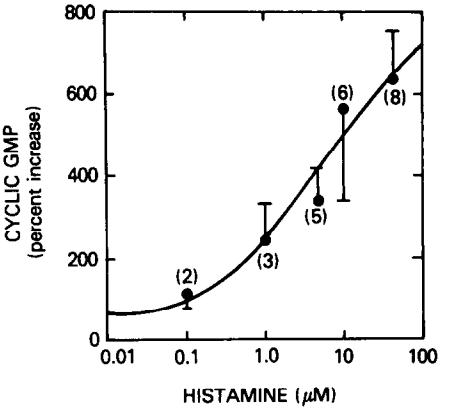

Fig. 5. Effect of adding increasing concentrations of histamine upon lung cyclic GMP content. Histamine in the concentrations listed was added to human lung fragments, and the cyclic GMP content was measured after 1.0 min. The control cyclic GMP content was 350 ± 30 fmoles/mg protein (n = 11). The data represent the mean ± S.E.M. percent increase in cyclic GMP content above the control. All values were significant at $P < 0.01$. The numbers in parentheses represent the number of separate experiments at each point.

< 10 nM [32]. However, Scatchard plots of initial individual binding experiments were nonlinear and had marked variation in the shape and in the bound axis intercept, suggestive of more than one binding site (Fig. 7). The calculated K_d ($1/K$) from these Scatchard plots, which was determined by attempting to fit a straight line to the plot of the bound to free ligand ratio versus the bound ligand concentration, was dependent on the range of radioligand concentration used in the individual experiments. That is, if the curvature were ignored or the concentration of radioligand were restricted to a narrow range, a straight line could be fit to the data; however, estimates of the K and R values were severely biased and therefore inaccurate. In addition, competition binding curves gave ED_{50} values in the micromolar range, which did not correspond to the K_d values initially estimated. Thus, the possibility of multiple classes of binding sites was entertained. However, estimates of the parameters for multiple binding sites were essentially indeterminant because of insufficient data from any one experiment (8–12 points). One of the most important features of the LIGAND computer program is that it enables the investigator to pool and fit data from multiple experiments [30].

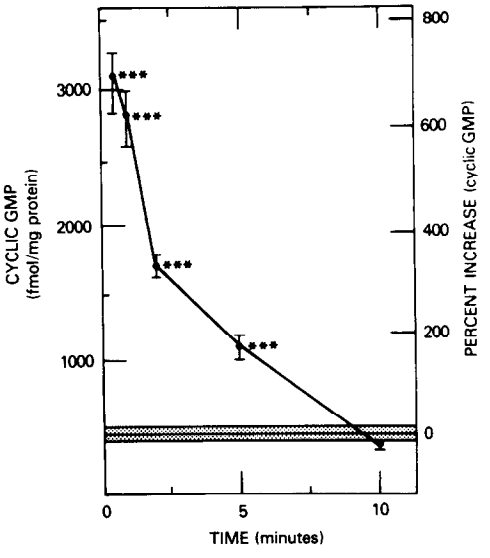

Fig. 4. Time course of the histamine-induced increase in cyclic GMP content of human lung tissue. The effect of 10 μM histamine in cyclic GMP is demonstrated. The shaded area represents the control cyclic GMP level of 450 ± 40 fmoles/mg protein. The data represent the pooled observations of three separate experiments. Key: (***) $P < 0.001$.

Table 3. Effect of pyrilamine on histamine-induced increases in cyclic GMP*

		Cyclic GMP (fmoles/mg protein)
Expt. A	Control	470 ± 300
	Histamine (30 μM)	1600 ± 600
	Pyrilamine (10 μM)	300 ± 30
	Histamine + pyrilamine	370 ± 100
Expt. B	Control	178 ± 30
	Histamine (50 μM)	440 ± 54
	Pyrilamine (10 μM)	150 ± 42
	Histamine + pyrilamine	220 ± 80
Expt. C	Control	460 ± 190
	Histamine (50 μM)	5100 ± 1200
	Pyrilamine (50 μM)	400 ± 200
	Histamine + pyrilamine	400 ± 220

* Lung fragments were incubated with the H-1 antagonist pyrilamine 20 min before histamine was added, and cyclic GMP was measured 1 min after the introduction of histamine. Pyrilamine effectively prevented the histamine-induced rise in cyclic GMP in all three experiments ($P < 0.05$).

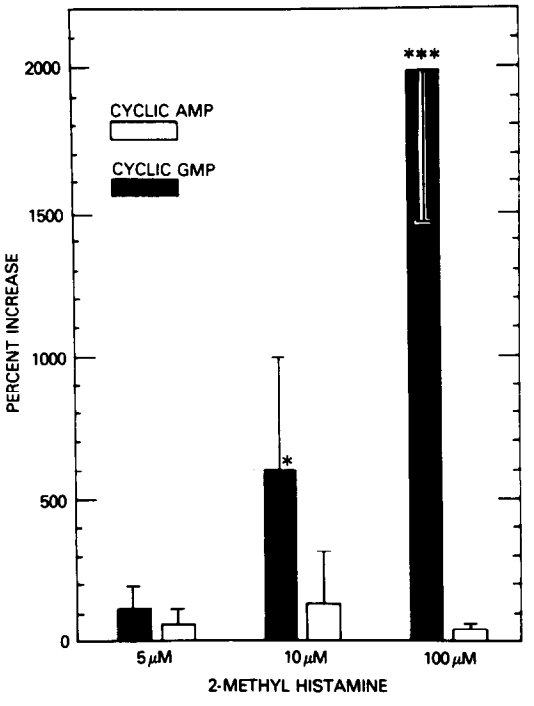

Fig. 6. Effect of adding increasing concentrations of 2-methyl histamine upon lung cyclic nucleotide content. 2-Methyl histamine in the concentrations listed was added to human lung fragments, and the cyclic GMP (closed bars) and cyclic AMP (open bars) levels were measured after 2.5 min. The data represent the mean ± S.E.M. percent increase in cyclic nucleotide content above the control for the pooled observations from six separate experiments. The control cyclic GMP content was 460 ± 190 fmoles/mg protein, and the control cyclic AMP was 7.6 ± 1.3 pmoles/mg protein. Key: (*) $P < 0.05$; and (***) $P < 0.001$.

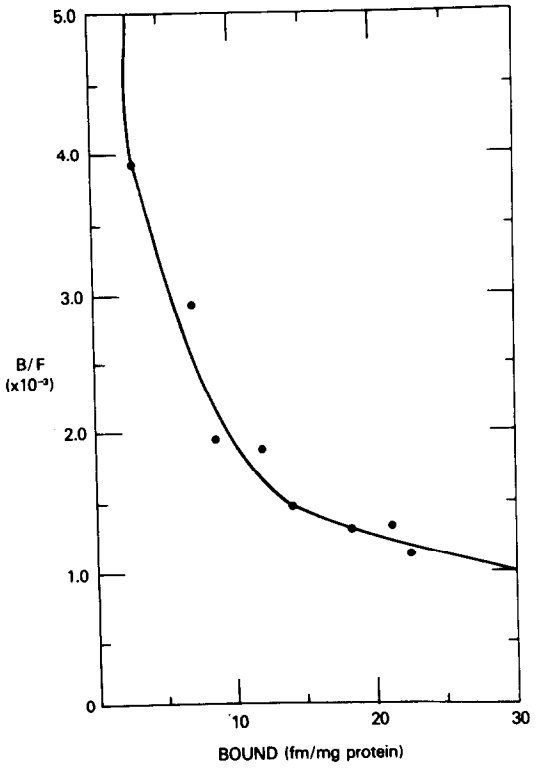

Fig. 7. Scatchard plot of the specific binding of [^{3}H]pyrilamine to human lung plasma membranes. The ratio of specifically bound over free [^{3}H]pyrilamine (B/F) is plotted as a function of specifically bound [^{3}H]pyrilamine. Even after normalization of the data for protein content and the subtraction of nonspecific binding estimated by the addition of excess unlabeled pyrilamine, the curve is nonlinear and suggestive of multiple classes of binding sites.

After pooling the data generated from fourteen different equilibrium binding studies (>250 points) employing a wide range of both hot and cold pyrilamine concentrations, we obtained an excellent fit for a model having three classes of binding sites (Table 1). The average scatter of a point around the fitted curve (Fig. 1) corresponded to only a ±18% error in the value of [Bound] for any given value of [Total]. The K values for all three classes of sites have only about a ±40–50% error. The R value for the highest affinity class of sites has about a ± 13% error, while those of the intermediate and low affinity classes of sites have errors of 50 and 40% respectively. The overall value for N is very well determined (± 9%) as were the correction factors for experiments 2–14 (± 7–11%). Since these parameter estimates have more nearly a log-normal than a normal distribution, the K and R values have standard errors less than the mean value by a factor of 2 on a log scale.

The fit involving three classes of sites is significantly better than fits involving only one or two classes of sites. When the "extra sum of squares" principle is employed, the use of only a single class of site increases the RMS from 18 to 36% (Table 1) with a corresponding F value of 155, which is highly significant. Assuming that there are only two classes of binding sites rather than three, the RMS is increased from 18 to 22% with a corresponding F value of 41 ($P < 0.001$). Hence, a model involving only one or two classes of sites is less acceptable statistically than that with three classes of sites. The kinetics of dissociation for [^{3}H]pyrilamine binding were also suggestive of three classes of sites.

Further evidence for the existence of multiple binding sites comes from the "logit-log" slopes of the drug competition curves which were consistently <1 for the drugs tested. In addition, the data for [^{3}H] pyrilamine displacement by D-chlorpheniramine, L-chlorpheniramine, and unlabeled pyrilamine could best be fit by a model involving three classes of sites.

It is readily apparent from the cyclic nucleotide data (Figs. 4–6, Table 3) that exogenous stimulation of human lung H-1 receptors with histamine or H-1 agonists resulted in increases in cyclic GMP, but not cyclic AMP. This rise in cyclic GMP occurred rapidly and was dependent on the concentration of agonist. These results confirm previous studies that also indicated that H-1 receptor stimulation in human lung results in a rise in cyclic GMP [22].

Whether one or more classes of binding sites is physiologically more significant than another

remains speculative at this point. Since the content of histamine in human peripheral lung is approximately 10 μg/g tissue, it is estimated that the release of histamine from mast cells may result in local concentrations around mast cells of 10 μM. As the concentration of histamine required to significantly elevate cyclic GMP levels is also in the micromolar range, it is plausible that the intermediate affinity site (K_d = 7 μM) may be the most physiologically important. However, these experiments employed intact lung fragments or cell membrane preparations from human peripheral lung, both of which are comprised of multiple cell types. Thus, it is possible that the three distinct binding sites reflect distinct binding characteristics of different cell types. Current investigations are being done to further characterize each of the three binding sites and to elucidate which cell types express these sites.

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