Biochemical Analysis of Specific Histamine H1 and H2 Receptors on Lymphocytes


It is increasingly clear that histamine mediates a variety of lymphocyte functions. Further understanding of these mechanisms requires a method for the analysis of histamine membrane receptors on the lymphocyte surface. We report now a biochemical technique for the identification and quantitation of specific histamine H1 and H2 receptors on lymphocytes. The method can be performed on small numbers of formaldehyde-fixed cells. The data this assay yields, together with that resulting from the flow cytometric analysis of histamine receptor distribution (a technique we have previously described), will be a powerful tool in the study of histamine mediation of lymphocyte function.

Histamine mediates a wide variety of physiologic and pathologic reactions through a membrane receptor system. Lymphocyte membrane receptors for histamine have been identified previously by a variety of techniques, including the binding of cells to histamine-coated beads or erythrocytes, and the effect of histamine on intracellular adenosine monophosphate (AMP). In addition, we have reported the solubilization, separation, and identification, by biochemical means, of specific histamine H1 and H2 receptors on calf thymus. More recently, we have described a flow cytometric technique that allows for the identification of histamine receptors on individual cells.

The study of histamine receptors on lymphocyte subpopulations may be a useful adjunct in formulating and understanding the contribution of immunologic mechanisms to the pathophysiology of certain conditions, such as atopic disease, neoplastic disease, histiocytosis-X, and autoimmune diseases, as well as other settings possibly characterized by imbalances in lymphocyte subpopulations.

However, the work of others concerning diseases that have been well correlated with abnormalities of receptor function suggest that it may not be sufficient to determine, by qualitative means, the number of receptor-bearing cells since receptor concentration and affinity may be equally important. A critical limitation, therefore, of the flow cytometric technique we have described is that it can only qualitatively assign each lymphocyte analyzed to the receptor-bearing positive or negative populations, when, in fact, a biochemical determination of the actual number, concentration, and affinity of the specific H1 and H2 histamine receptors may be necessary.

MATERIALS AND METHODS

Preparation and Fixation of Lymphocytes

Lymphocytes were prepared by Ficoll-Hypaque fractionation of peripheral blood collected in preservative-free heparin. After centrifugation, cells were fixed by resuspension in phosphate-buffered normal saline (PBS) containing 1% formaldehyde and stored at 4°C until use.

Binding of 3H-Histamine to Fixed Cell

The fixed cells to be assayed were washed twice in PBS and finally resuspended in PBS containing 2 mmole CaCl2 and 1 mmole MgSO4 (PBS/Ca-Mg) at a concentration of 2 × 107 cells/ml. Twenty-five microliters of the cell suspension was placed in each well of a microtiter plate (Multi-well Microtitration plate, Linbro Scientific Co., Handen, Conn.) to which was added 3H-histamine (specific activity 6.1–8 Ci/m mole, New England Nuclear, Boston, Mass.) to a final concentration ranging from 3 × 10−5 M to 3 × 10−3 M; each concentration was done in triplicate wells.

The cells were incubated in the 3H-histamine alone, or in the presence of 5 × 10−5 M (final concentration) unlabeled competing histamine (histamine dihydrochloride, Sigma Chemical Co., St. Louis, Mo.). PBS/Ca-Mg was added to wells to make a final volume of 50 μl. The cells were incubated at either 4°C, 20°C, or 37°C for various times.

At the conclusion of the incubation, the plates were harvested on a Tittertek cell harvester (Flow Laboratories, Rockville, Md.), using filter mats that had been prewashed with 5 × 10−3 M unlabeled histamine. The washing of the plate during the actual harvesting was done with water containing 5 × 10−3 M unlabeled histamine, stored at 4°C until use. The filter mats were dried thoroughly, and samples were counted using Econofluor (New England Nuclear) in a Beckman LS7500 scintillation counter. The triplicate wells were averaged.

Determination of Specific H1 and H2 Type Receptors

Cells were prepared as above and incubated for 45 min at 37°C with 10−3 M 3H-histamine, either alone or in the presence of a 100-fold greater concentration of the H1 receptor antagonist diphenhydramine HCl (Sigma Chemical Co.) or the H2 receptor diphenhydramine HCl (Sigma Chemical Co.).
antagonist cimetidine (Smith Kline and French Laboratories, Philadelphia, Pa.). At the conclusion of the incubation the amount of bound $^3$H-histamine was determined.

**Calculation of Specific Total Histamine, H1 and H2 Receptor Numbers**

The amount of $^3$H-histamine specifically bound to histamine receptors was calculated as follows. Total specifically bound $^3$H-histamine was the difference in the radioactivity between incubations performed in the absence and presence of $5 \times 10^{-7} M$ unlabeled competing histamine. $^3$H-histamine specifically bound to H1 and H2 receptors was the difference between total bound histamine from incubations done in the absence and presence of a 100-fold greater concentration of the respective antagonist. Knowledge of the specific activity of the $^3$H-histamine and the number of cells present in the reaction, together with the assumption that each receptor binds only one molecule of histamine, enables the calculation of receptor number per cell.

**Effect of Ambient Histamine on Receptor Number Determination**

To eliminate the possibility that variations in receptor number might be due to the concentration of circulating histamine in the donor at the time of blood collection, the following experiment was done. Peripheral blood was collected as above and incubated in the absence or presence of $10^{-5} M$ unlabeled histamine dihydrochloride for 2 hr at 37°C prior to lymphocyte preparation and receptor number determination. Receptor number was then determined in each group as described above.

**RESULTS**

**Specific Binding as a Function of $^3$H-Histamine Concentration**

Cells were incubated at 4°C, 20°C, and 37°C for varying periods of time ranging from 1 to 90 min with varying concentrations of $^3$H-histamine, either alone (total binding) or in the presence of $5 \times 10^{-7} M$ competing unlabeled histamine (nonspecific binding). As shown in Fig. 1, specific binding (total minus nonspecific) was saturated at approximately $8 \times 10^{-6} M$ $^3$H-histamine. Maximum binding of the receptors was achieved with incubation at 37°C for 45 min.

Incubation at 20°C demonstrated fewer specific sites bound, while there was no specific binding with incubation at 4°C.

**Specific H1 and H2 Receptor Binding**

Cells were incubated with $10^{-5} M$ $^3$H-histamine for 45 min at 37°C either alone or in the presence of a 100-fold higher concentration of either the H1 receptor antagonist diphenhydramine or the H2 receptor blocker cimetidine, thus ensuring complete blocking of the respective H1 or H2 receptor. The number of specific H1 or H2 receptor sites was calculated as described above, and the results for a typical experiment are given in Table 1.

**Table 1. Determination of Specific Histamine Receptors**

<table>
<thead>
<tr>
<th>Incubation Condition</th>
<th>$^3$H-Histamine Bound/10⁶ Cells</th>
<th>Number of Specific Receptors/Cell*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Incubation in $^3$H-histamine only</td>
<td>319</td>
<td>170,000</td>
</tr>
<tr>
<td>B. Incubation in $^3$H-histamine in presence of unlabeled histamine</td>
<td>36</td>
<td>70,000</td>
</tr>
<tr>
<td>C. Incubation in $^3$H-histamine in presence of diphenhydramine (H1 antagonist)</td>
<td>202</td>
<td>121,000</td>
</tr>
<tr>
<td>D. Incubation in $^3$H-histamine in presence of cimetidine (H2 antagonist)</td>
<td>118</td>
<td></td>
</tr>
</tbody>
</table>

1. Total specific histamine receptors (calculated from A - B) 170,000
2. Specific H1 histamine receptors (calculated from A - C) 70,000
3. Specific H2 histamine receptors (calculated from A - D) 121,000

The upper half of the table gives the number of fmole of $^3$H-histamine bound/10⁶ cells assayed. Using those determinations, the actual number of receptors (total histamine, specific H1, and specific H2) are calculated, as explained in the text.

*When the assay was performed on an identical aliquot of cells previously incubated in $10^{-8}$ ambient histamine (see text), the results were ± 2% of the data shown here.
HISTAMINE RECEPTORS ON LYMPHOCYTES

Effect of Ambient Histamine

Since it was possible that variations in receptor number between patients would reflect endogenous ambient histamine concentrations in the blood that were binding specific histamine sites at the time of blood drawing (autoblockade), we took samples of whole blood, divided them into two portions, and incubated each for 2 hr at 37°C. One aliquot of the sample was incubated without further preparation, while the other aliquot had unlabeled histamine added to a final concentration of 10^{-5}M. At the end of the incubation time the cells were processed as above and the amount of specific total histamine, H1 and H2 receptor sites determined. The results did not demonstrate any difference in the number of receptors present between these two preparations, therefore indicating that differences in receptor number reflect actual variation on cell surface and not variations in the ambient histamine present in the blood from which the cells are collected (Table 1).

DISCUSSION

A variety of lymphocyte functions appear to be mediated by the binding of ligands to surface receptors. We have previously reported a flow cytometric technique for the analysis and identification of histamine receptors on lymphocytes. That method determines the distribution of the receptor in a given cell population by assigning each cell analyzed to receptor-bearing positive or negative subsets. However, this flow cytometric technique is limited, in that it does not allow for the determination of receptor concentration or affinity. These values might prove very important in the understanding of histamine receptor function.18,19

We report a biochemical technique that does allow for the determination of receptor concentration (number of receptors/cell) and affinity (binding kinetics). It is based on certain principles established in our previous biochemical work with histamine receptors.7 This new assay has certain distinct advantages. It can be performed on small numbers of fixed cells (5 x 10^6), so that samples could be collected and stored over time for simultaneous assay. Methodologically, it is convenient and simple. Furthermore, the biochemical technique we described previously for calf thymus7 would be impossible to adapt to lymphocytes on a routine basis, since it requires a considerably larger number of cells (3 x 10^8) and is much more complex with both ultracentrifugation and gel filtration steps.

The study of histamine effect on lymphocyte function is becoming increasingly important. A fuller understanding of these mechanisms requires techniques that determine the distribution and quantitation of specific histamine H1 and H2 receptors. Our previously published flow cytometric technique, when used in combination with the biochemical technique reported here, provides a powerful tool for the full analysis of histamine receptors on various lymphocyte subpopulations. The use of these two methods will allow for the determination of the percentage of a given population that bears either H1 or H2 type receptors, the number of receptors/cell in that total population, and then, by simple calculation, the number of specific receptors/receptor-bearing cell. Finally, it should be emphasized that this technique is applicable to any tissue from which a single cell population can be made.

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REFERENCES

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ME Osband, EB Cohen, BR Miller, YJ Shen, L Cohen, L Flescher, AE Brown and RP McCaffrey