Histamine Release Induced by Human Leukocyte Lysates: Effect of Metabolic Inhibitors and Carbohydrates

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The effects of metabolic inhibitors and carbohydrates on histamine release induced by human leukocyte lysates have been studied to gain an understanding of the mechanism of histamine release in this system. Dinitrophenol produced 80% inhibition of histamine release at a concentration of $10^{-4}$ M. Iodoacetic acid inhibited release to a lesser extent, and deoxyglucose had relatively little effect. Colchicine was a potent inhibitor of release and produced 88% inhibition at a $10^{-4}$ M concentration. Puromycin and cycloheximide had essentially no effect on histamine release. Various sugars inhibited release with mannitol and glucose, producing greater than 50% inhibition at $10^{-4}$ M concentrations. Other sugars inhibited histamine release to a lesser degree with the following order of potency: mannitol > glucose > sucrose > galactose > fructose. These studies suggest that intact, metabolically active cells are required for histamine release induced by human leukocyte lysates and that a secretory process is involved. The results also provide information on the possible nature of the histamine-releasing factor(s) in human leukocyte lysates.

In the preceding paper (7) we presented some of the characteristics of the leukocyte histamine release reaction induced by human leukocyte lysates. The characteristics presented suggest that this system is unlike those previously described. In the present study, we have examined the effects of various metabolic inhibitors and carbohydrates on histamine release induced by human leukocyte lysates. Histamine release was reduced in the presence of inhibitors of oxidative metabolism and glycolysis, but inhibitors of protein synthesis had little effect. The system was also inhibited by colchicine and various sugars. These findings bear greatly on the mechanism of histamine release and on the possible nature of leukocyte lysate histamine-releasing factors.

MATERIALS AND METHODS

Leukocytes and buffers. Leukocytes were collected from the peripheral blood of normal human donors by dextran sedimentation and prepared as previously described (6). Cells were washed in tris(hydroxymethyl)aminomethane (Tris) A (10) and suspended in Tris ACM (10) for histamine release assays. Leukocyte lysates were prepared by sonic treatment as previously described (6).

Inhibitors. Stock solutions of 2,4-dinitrophenol, iodoacetic acid, 2-deoxy-D-glucose, cycloheximide, puromycin (all obtained from Nutritional Biochemical Co., Cleveland, Ohio), actinomycin D (Cosmegen, Merck, Sharp & Dohme, Rahway, N.J.), and colchicine (Sigma Chemical Co., St. Louis, Mo.) were prepared in Tris ACM and stored at -70°C. The stock solutions were diluted in Tris ACM to the desired concentrations just before use. Carbohydrate solutions were made fresh daily in Tris ACM.

Histamine release assay. Ten million leukocytes were incubated for 30 min at 38°C in 3.5 ml of Tris ACM containing 0 to $10^{-4}$ M concentrations of various test agents. After equilibration, leukocyte lysate in Tris ACM containing an appropriate concentration of test agent was added to the leukocyte suspensions and incubated for 60 min at 38°C. The final concentration of lysate was 0.25 to 1.0 µg of protein per ml, which provided optimal histamine release. The histamine released into the incubation mixture supernatant fluids was assayed fluorimetrically, as previously described (10).

Control incubations not containing test agent were included in each experiment, and percentage of inhibition values were calculated based on these controls. Assays of standard histamine solutions containing the maximal concentrations of the inhibitors used in these studies demonstrated that these agents did not interfere with the histamine assay with the exception of colchicine, which was removed by the method of Levy and Carlton (8). Spontaneous histamine release
in the absence of leukocyte lysate was always less than 10% of the total available histamine, and the presence of inhibitors did not alter this value.

RESULTS

Effects of energy metabolism inhibitors. Exposure of leukocytes to dinitrophenol during incubation with leukocyte lysate strongly inhibited histamine release (Fig. 1). Approximately 10^{-4} M dinitrophenol produced 50% inhibition of histamine release, and 80% inhibition was obtained with 10^{-3} M dinitrophenol (Table 1).

The effects of glycolysis inhibitors on histamine release were also examined. Iodoacetic acid had a biphasic effect on histamine release. The maximal inhibition obtained was 55% at a concentration of 10^{-3} M iodoacetic acid (Table 1). However, this agent had no effect on histamine release at a concentration of 10^{-4} M, and 10^{-3} M iodoacetic acid inhibited release by about 25%.

Deoxyglucose, which is an analogue of glucose and thereby inhibits its metabolism, was a very weak inhibitor of leukocyte lysate-induced histamine release. The highest concentration of deoxyglucose tested, 10^{-3} M, inhibited histamine release by only 25% (Table 1).

Effects of protein synthesis inhibitors. Leukocytes were exposed to varied concentrations of puromycin and cycloheximide for 30 min at 38 C and then incubated with lysate in the continued presence of the inhibitors. The maximal inhibition of histamine release was only 8% with 10^{-4} M puromycin and 14% with 10^{-3} M cycloheximide (Table 1). Actinomycin D was also examined, and it had no effect on histamine release. Therefore, inhibition of leukocyte protein synthesis had little or no effect on lysate-induced histamine release.

Histamine release inhibition by colchicine. The effect of colchicine on lysate-induced histamine release was examined to evaluate the role of microtubules in this system. Leukocytes were incubated in the presence of colchicine in concentrations ranging from 10^{-2} to 10^{-7} M. After 30 min, leukocyte lysate was added to the incubation mixtures and histamine release was determined. Colchicine was a potent inhibitor of histamine release. Maximal inhibition was 88% at 10^{-3} M (Table 1), and a concentration of approximately 2 \times 10^{-4} M produced 50% inhibition.

Histamine release inhibition by carbohydrates. The effect of glucose on histamine release induced by human leukocyte lysates was examined with the thought that it might play a permissive role in the release process because energy metabolism is required. Leukocytes were incubated in the presence of 5 \times 10^{-3} to 10^{-4} M concentrations of glucose and then incubated with lysate in the continued presence of glucose. Glucose inhibited histamine release, and a linear dose-response relationship was obtained for the concentrations tested (Fig. 2). The maximal inhibition obtained was 76% at 5 \times 10^{-3} M glucose.

The inhibitory effect of glucose on lysate-induced histamine release was further analyzed by adding glucose (10^{-3} M final concentration) to leukocyte suspensions at 5, 10, or 15 min after the addition of leukocyte lysate. Samples were removed at intervals up to 60 min and assayed for histamine release. Glucose interrupted histamine release when added at any time up to 15 min after the initiation of release (Fig. 3).

Other sugars were tested for ability to inhibit histamine release, and all those tested had inhibitory activity at 10^{-3} M concentrations (Table 2). Mannitol had the greatest inhibitory effect, followed by glucose, sucrose, galactose, and fructose. Another metabolizable carbohydrate, sodium succinate, had essentially no effect on histamine release in this system.

**Table 1. Effects of antimetabolites on histamine release induced by human leukocyte lysates**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration (M)</th>
<th>Inhibition (%)</th>
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</thead>
<tbody>
<tr>
<td>Dinitrophenol</td>
<td>10^{-3}</td>
<td>80</td>
</tr>
<tr>
<td>Iodoacetic acid</td>
<td>10^{-3}</td>
<td>55</td>
</tr>
<tr>
<td>Deoxyglucose</td>
<td>10^{-3}</td>
<td>25</td>
</tr>
<tr>
<td>Puromycin</td>
<td>10^{-4}</td>
<td>8</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>10^{-3}</td>
<td>14</td>
</tr>
<tr>
<td>Colchicine</td>
<td>10^{-3}</td>
<td>88</td>
</tr>
</tbody>
</table>

**Fig. 1. Inhibition of leukocyte lysate-induced histamine release by dinitrophenol.** Leukocytes were incubated in the presence of varied concentrations of dinitrophenol. The leukocytes were challenged with leukocyte lysate, and the percentage of inhibition of histamine release was determined.
interfere with leukocyte lysate-induced histamine release, whereas inhibitors of protein synthesis have no effect.

Dinitrophenol, an inhibitor of oxidative phosphorylation, was one of the most potent inhibitors of histamine release (Table 1), which suggests that oxidative energy metabolism is required for leukocyte lysate-induced histamine release. Iodoacetic acid also inhibited histamine release, suggesting that glycolysis may also be important. However, deoxyglucose had relatively little effect on the release reaction, and if glycolysis is required for the histamine release reaction, deoxyglucose would be expected to have a more potent effect on release. As noted by Schild (16), iodoacetic acid may have nonspecific effects on sulphydryl-containing proteins other than glycolytic enzymes. The biphasic nature of the inhibition by iodoacetic acid observed in the present study may indicate that this agent interferes with histamine release by interaction with proteins or enzymes involved with the release mechanism rather than by inhibition of glycolysis. Therefore, the effects of these metabolic inhibitors suggest that intact, metabolically active cells are required for leukocyte lysate-induced histamine release, and it is likely that oxidative metabolism is most important in the release reaction.

Several other histamine release systems have been shown to require metabolically active cells (9, 14, 15, 16). Histamine release induced by antigens is inhibited by iodoacetic acid and deoxyglucose but not by dinitrophenol, suggesting that glycolysis is the important energy source in this type of histamine release. However, histamine release induced by adenosine triphosphate (ATP) (2), cationic protein (15), 48/80 (17), or dextran (1) all require oxidative metabolism because they are inhibited by dinitrophenol. Since oxidative metabolism seems to be the most important energy source for lysate-induced histamine release, it most closely resembles the nonimmunological systems mentioned above in this respect.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol</td>
<td>73</td>
</tr>
<tr>
<td>Glucose</td>
<td>59</td>
</tr>
<tr>
<td>Sucrose</td>
<td>39</td>
</tr>
<tr>
<td>Galactose</td>
<td>35</td>
</tr>
<tr>
<td>Fructose</td>
<td>27</td>
</tr>
<tr>
<td>Succinate</td>
<td>7</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Our previous studies have demonstrated that lysates of human leukocytes release histamine from intact human leukocytes in vitro (6). This system involves a specific, complement-independent, noncytotoxic reaction which proceeds optimally under physiological conditions (7). The present study demonstrates that metabolic inhibitors and mono- and disaccharides...
Lysate-induced histamine release was not inhibited by cycloheximide or puromycin, suggesting that protein synthesis is not required in the release reaction. This indicates that the machinery for histamine release exists in a preformed state and is activated by lysate during the release reaction. This is in accord with the hypothesis proposed by Mongar and Schild (13), that histamine release is modulated by an enzyme-like system which is activated by inducers of histamine release.

Histamine release in other systems is thought to involve a secretory process (A. G. Osler, Fed. Proc., 1969, p. 1729-1736). The inhibition of histamine release by colchicine suggests a similar process for our system. Colchicine has been shown to disrupt the microtubule apparatus in human leukocytes (11), and microtubules may play a role in secretory processes (18). Therefore, like the allergic (8) and 48/80 (E. Gillespie et al., Fed. Proc., 1967, p. 786) histamine-releasing systems, human leukocyte lysate-induced histamine release may involve a secretory process.

Histamine release has been induced by a variety of substances including antigens (9, 14, 16), cationic protein (15), polyamines (4), compound 48/80 (17), ATP (2), and dextran (1). A comparison of these systems to the human leukocyte lysate system may provide suggestions about the nature of the lysate histamine-releasing factor. In this regard, the inhibitory effects of mannitol, glucose, and other sugars on lysate-induced histamine release are particularly interesting.

Dextran-induced histamine release from rat mast cells is inhibited by glucose and related sugars (1) by a competitive mechanism (3). Control experiments in our previous studies indicated that the histamine-releasing activity in leukocyte lysates was not due to the dextran used in preparation of the leukocytes (6). However, the inhibitory effects of sugars in our system do suggest that the histamine-releasing factor in human leukocyte lysates may be similar to dextran or contain carbohydrate moieties which are important for activity. More definitive evidence on the nature of the histamine-releasing activity must await purification of the active factor involved, but these studies may provide useful guidelines for future investigation.

ACKNOWLEDGMENTS

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LITERATURE CITED


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