Anticoagulant substance released from human lung mast cells by stimulation with anti-IgE or Ca-ionophore A23187.

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Abstract

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KEYWORDS: human lung mast cells, heparin, anticoagulant activity, anti-IgE, Ca-ionophore

*PMID: 2438904 [PubMed - indexed for MEDLINE]
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Anticoagulant Substance Released from Human Lung Mast Cells by Stimulation with Anti-IgE or Ca-Ionophore A23187

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Basophils and mast cells contain proteoglycans (PGs) responsible for the metachromatic staining properties of the granules of these cells (1). PGs participate in the granule storage of histamine and other chemical mediators (2). Until recently, little was known about the physiologic roles of PGs which were simultaneously released with histamine during degranulation of both kinds of cells in IgE-dependent allergic reactions. The present experiments were performed to characterize the anticoagulant substance released from human lung mast cells stimulated with anti-IgE or Ca-ionophore A23187.

Anticoagulant activities were determined by the amidolytic methods of Lasser et al. using the chromogenic substrates S2238 and S2222 (AB Kabi, Sweden) with porcine intestine mucosal heparin (Up John, USA) as a standard (3). The heparin concentration was detectable in the range of 0.005 to 0.1 U/ml using S2238 in an anti-thrombin assay and S2222 in an anti-Factor Xa assay. Metachromatic substances were measured by a method modified from that of Jaques et al. (4). Histamine was quantitated by an automated spectrofluorometric histamine analysis system (Technicon) (5).

Human lung mast cells were obtained from normal part of human lung tissue (10-20g) removed surgically with carcinoma, and were isolated by the method of Caulfield et al. (6) with minor modifications (7). Isolated mast cells (5.5×10⁶-1.7×10⁷ cells, purity 11-35%) were passive-sensitized for 2 h at 37°C with high IgE serum from an allergic patient at the final IgE concentration of 1,000U/ml. Cells were washed three times and resuspended in 1 ml of Tyrode’s buffer with 1/25 final volume of anti-human IgE rabbit serum (Behringwerke AG, West Germany). Isolated mast cells were also stimulated with Ca-ionophore A23187 (Sigma, USA) at the final concentration of 1 µg/ml. After incubation at 37°C for 30 min with anti-IgE or for 10 min with A23187, cells were sedimented at 500g for 5 min at room temperature, and the supernatants were removed. Cell pellets
were sequentially frozen and thawed six times in 1 ml of calcium-magnesium-free Tyrode’s buffer. Anti-thrombin activity, and amounts of histamine and metachromatic substance in the supernatant and the frozen-thawed extract of cell pellet were measured. The supernatants were also incubated with an equal amount of heparinase, chondroitin-ABC lyase or chondroitin-AC lyase at 37°C for 60 min. The anti-thrombin activity in 50 μl of thus treated supernatant was measured with 10 μl of human plasma, 150 μl of thrombin (1.0 U/ml) and 190 μl of 0.2 M Tris-HCl buffer pH 8.4 using th chromogenic substrate S2238 (H-D-Phe-Pip-Arg-p-nitroanilide). The activity of remaining thrombin was estimated by measuring p-nitroaniline from S2238 photometrically at 405 nm.

Significant release of anticoagulant substance from human lung mast cells, with simultaneous release of histamine, occurred after stimulation with anti-IgE or Ca-ionophore (Table 1). Anti-thrombin activity induced by anti-IgE was 33.3 ± 5.8% of the total, very similar to the release of metachromatic substances (32.6 ± 9.4%). Anti-thrombin activity of 0.026 ± 0.022 U/5 × 10⁶ cells was detected after stimulation with anti-IgE, and 0.045–0.093 U/5 × 10⁶ cells with Ca-ionophore. Anti-factor Xa activity of 0.003 and 0.071 U/5 × 10⁶ cells was also detected in the supernatants of two preparations of human lung mast cells with anti-thrombin activity of 0.008 and 0.057 U/5 × 10⁶ cells, respectively. This anticoagulant activity was inhibited by adding Polybrene (Sigma, USA) at a final concentration of 4 μg/ml to the reaction mixture of the anti-thrombin assay (data not shown). As shown in Fig. 1, anti-thrombin activity was lost after treatment with heparinase (Seikagaku Kogyo, Japan) at final concentrations of 1.25 × 10⁻¹ to 12.5 U/ml, but not after treatment with chondroitin-ABC lyase (Seikagaku Kogyo, Japan) or chondroitin-AC lyase (Seikagaku Kogyo, Japan) at final concentrations of 1.25 × 10⁻³ to 1.25 U/ml. The anti-thrombin activity of 1 mg of chondroitin sulfate B (Sigma, USA) in the presence of human plasma was blocked using chondroitin-ABC lyase at the concentration which did not inhibit the anti-thrombin activity from human lung mast cells. No detectable release of substance with anti-thrombin activity from human basophils was observed by stimulation with either anti-IgE or Ca-ionophore (Table 1).

Significant release of anticoagulant substance with simultaneous histamine release from human lung mast cells was found after treatment with anti-IgE and Ca-ionophore.

Table 1 Percent release of anticoagulant substance, metachromatic substance and histamine from human lung mast cells by stimulation with anti-IgE or Ca-ionophore A23187

<table>
<thead>
<tr>
<th>Cells and stimulant</th>
<th>Anti-thrombin activity (S2238)</th>
<th>Metachromatic substance</th>
<th>Histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung mast cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-IgE (1000 U/ml)</td>
<td>33.3 ± 5.8</td>
<td>32.6 ± 9.4</td>
<td>11.2 ± 5.1</td>
</tr>
<tr>
<td>A 23187 (1 μg/ml)</td>
<td>20.9–28.8</td>
<td>35.9–50.0</td>
<td>14.7–18.9</td>
</tr>
<tr>
<td>Basophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-IgE (1000 U/ml)</td>
<td>Not detected</td>
<td>37.8 ± 5.2</td>
<td>7.8 ± 0.8</td>
</tr>
<tr>
<td>A 23187 (1 μg/ml)</td>
<td>Not detected</td>
<td>Not assayed</td>
<td>Not assayed</td>
</tr>
</tbody>
</table>

α: The percentage of release of mediators was calculated by the formula:

\[
\% \text{ release} = \frac{\text{content of mediators in supernatant}}{\text{content of mediators in supernatant and sediment}} \times 100,
\]

and net percentage of release was determined by correcting spontaneous release from control cells processed in parallel. Results are expressed as mean ± SE.

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Anticoagulant Substance Release from Mast Cells

The percent release of substance with anti-thrombin activity by anti-IgE was very similar to that of metachromatic substances. This anticoagulant activity was detectable not only using the chromogenic substrate S 2238 in an anti-thrombin assay but also using S2222 in an anti-Factor Xa assay. The activity was lost by heparinase, and not by chondroitin-ABC lyase or chondroitin-AC lyase. This anticoagulant activity also became undetectable after adding Polybrene to the reaction-mixture. It has been reported that a suitable amount of Polybrene prevents the formation of thrombin-antithrombin III complex in the presence of heparin, but not in the presence of chondroitin sulfate B (dermatan sulfate) (8). Therefore, it is suggested that the anticoagulant substance released from lung mast cells was heparin. The heparin released together with histamine from human lung mast cells may participate in modifying IgE-mediated allergic reactions in inflammed tissues to prevent clot formation.

References

1. Lagunoff D: Analysis of dye binding sites in the mast cell granules. Biochemistry (1974) 13, 3982-


Received: July 28, 1986
Accepted: February 3, 1987

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