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Histone Shock and The Relation between Histone and Plasmin

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The relation between protease and nucleoprotein (histone) is described on the point of anaphylactoid shock in guinea pigs. Histone caused the anaphylactoid shock in guinea pigs, but histone with plasmin incubated for twenty-four hours did not. Histone released the histamine significantly from the mast cells.

The mechanism of anaphylactic and anaphylactoid phenomenon has been done experimenting and many theories have been advocated. Among them protease theories (1, 2) have called much attention to the possibility of explaining histamine release as the resulting from the activation of proteolytic enzymes. Many evidences have been accumulated that the protease activity increases on the antigen-antibody reaction (3-6), tissue injuries (7, 8) and other (9). Plasmin, however, has an important weak point, because of less histamine release from the tissue (10, 11).

The object of the work which is reported in this paper was to investigate histone shock on guinea pigs and the relation between plasmin and histone.

MATERIALS AND METHODS

Guinea pigs of both sexes and weighing between 120 and 400g were used for histone shock.

Wistar rats of both sexes and weighing between 120 and 150g were used for collecting mast cells. The mast cells were isolated by a method almost similar to that described by Glick, Bonting and DenBoer (12). The animals were bled by decapitation and 3 ml of heparinized (50 μg/ml) Hanks’ balanced salt solution were injected into the abdominal cavity and gentle massage of the gut through the abdominal wall was performed for about 1.5 minutes. The abdominal wall was then incised along the mid ventral line and the fluid from the abdominal cavity collected with a pipette. This was carefully layered above 3 ml of sucrose working solutions which was allowed to reach room temperature displaced from cold room where it had been set up twenty-four hours earlier. The
solution contained mast cells was diffused the interface by gentle stirring with a thin glass rod and then centrifuged for a total of 5 minutes, gradually increasing the speed to a maximum of 110 xg and then gradually reducing to zero.

The mast cells layer was removed with a pipette and each ml of mast cell suspension in sucrose solution was added about 3 ml of distilled water and centrifuged for 5 minutes at 452 xg. Most of the supernatant fluid were removed and replaced with Hanks' balanced salt solution.

Histone (Nutritional Biochemicals Corp., U. S. A.) was dissolve in 1/50 N HCL and neutralized by 1/50 N NaOH.

Human plasmin was prepared from human serum adding streptokinase and the activity was estimated by measuring the hydrolysis of a synthetic substrates: p-toluylsufonyl-L-arginine-methyl ester (TAMe) and benzoyl-L-arginine ethyl ester (BAEe) purchased from MANN Research Laboratories, New York. On hydrolysis, The carboxylic acid became free, displaced CO$_2$ from the bicarbonate buffer and CO$_2$ was measured manometrically in a WARBURG apparatus.

Hanks' balanced salt solution which contained mast cells was incubated for 5 minutes in a water-bath (37° C) after adding histone and centrifuged. After centrifuge the deposit, which was replaced by equivalent of Hanks' balanced salt solution was heated in a boiling water-bath for 5 minutes. Histamine in both solution was measured by using bioassay on the guinea pig's ileum in tyrode solution which contained 1μ/ml of atropine.

RESULTS

Histone ranging from 10 to 5 mg was injected intravenously to guinea pigs. All of the guinea pigs received histone showed the symptom of anaphylactoid shock (itching, dyspnea, etc.) and six cases

<table>
<thead>
<tr>
<th>No.</th>
<th>Weight g</th>
<th>Histone mg</th>
<th>Shock Symptom</th>
<th>Death or Living</th>
<th>Pathological Finding of Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120</td>
<td>10</td>
<td>jump, dyspnea, falled down</td>
<td>died</td>
<td>emphysema (+)</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>10</td>
<td>jump, dyspnea, falled down</td>
<td>died</td>
<td>emphysema (+)</td>
</tr>
<tr>
<td>3</td>
<td>260</td>
<td>10</td>
<td>urination, jump, dyspnea, falled down</td>
<td>died</td>
<td>emphysema (+)</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>5</td>
<td>urination, itching</td>
<td>lived</td>
<td>emphysema (+)</td>
</tr>
<tr>
<td>5</td>
<td>230</td>
<td>8</td>
<td>dyspnea, falled down</td>
<td>died</td>
<td>emphysema (+)</td>
</tr>
<tr>
<td>6</td>
<td>240</td>
<td>9</td>
<td>falled down, dyspnea</td>
<td>died</td>
<td>emphysema (+)</td>
</tr>
<tr>
<td>7</td>
<td>350</td>
<td>8</td>
<td>dyspnea, falled down</td>
<td>died</td>
<td>emphysema (+)</td>
</tr>
<tr>
<td>8</td>
<td>400</td>
<td>8</td>
<td>dyspnea</td>
<td>lived</td>
<td>emphysema (+)</td>
</tr>
</tbody>
</table>

*± --- medium, + --- slight
Table 2
Histamine release from the mast cells by adding histone

<table>
<thead>
<tr>
<th>Agents</th>
<th>Concentration</th>
<th>Histamine in supernatant after addition</th>
<th>Histamine remaining in deposit</th>
<th>Total</th>
<th>Percentage histamine released</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histone</td>
<td>10 mg/ml, 1 ml to 1 ml</td>
<td>11.5 γ/ml</td>
<td>4.5 γ/ml</td>
<td>16.0 γ/ml</td>
<td>71.8%</td>
</tr>
<tr>
<td>Control (Hanks’ balanced salt solution)</td>
<td>1 ml to 1 ml</td>
<td>1.0</td>
<td>15.0</td>
<td>16.0</td>
<td>6.25</td>
</tr>
</tbody>
</table>

Fig. 1

![Graph showing histamine release](image)

H - Histamine
1 - Supernate of mast cells suspension after adding histone
① - Deposit of mast cell suspension after adding histone
2 - Supernate of mast cells suspension after adding Hank’ balanced salt solution
② - Deposit of mast cell suspension after adding Hank’ balanced salt solution

Among the eight died after showing anaphylactoid symptom. All of the cases were decapitated after showing shock revealed the emphysema of the lung (Table 1).

10 mg of histone added to 1 ml of mast cell suspension released 11.5 γ/ml of histamine in the supernate and 4.5 γ/ml of histamine remained in the deposit. Consequently, histamine release from one ml of mast cell suspension by the histone was 7.0 γ/ml. Hanks’ balanced salt solution instead of histone to mast cells released histamine 1.0 γ/ml and
Table 3
Effect of plasmin to histone causing shock on guinea pig

<table>
<thead>
<tr>
<th>Kind of Injection</th>
<th>Weight</th>
<th>Amount</th>
<th>Shock Symptom</th>
<th>Death or Living</th>
<th>Pathological Finding of Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>plasmin plasmin</td>
<td>g</td>
<td>mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>370</td>
<td>7</td>
<td>7</td>
<td>urination, acceleration of breathing</td>
<td>lived</td>
<td>emphysema (+)*</td>
</tr>
<tr>
<td>400</td>
<td>10</td>
<td></td>
<td>cry, acceleration of breathing</td>
<td>lived</td>
<td>emphysema (+)</td>
</tr>
<tr>
<td>histone histone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>350</td>
<td>8</td>
<td>8</td>
<td>dyspnea, falled down</td>
<td>died</td>
<td>emphysema (+++)</td>
</tr>
<tr>
<td>400</td>
<td>8</td>
<td></td>
<td>Urination, dyspnea</td>
<td>lived</td>
<td>emphysema (+++)</td>
</tr>
<tr>
<td>plasmin and histone plasmin and histone</td>
<td></td>
<td></td>
<td>itching</td>
<td>lived</td>
<td>emphysema (-)</td>
</tr>
<tr>
<td>320</td>
<td>plasmin 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>histone 8</td>
<td></td>
<td></td>
<td>itching</td>
<td>lived</td>
<td>emphysema (-)</td>
</tr>
<tr>
<td>plasmin 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>histone 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>350</td>
<td>histone 8</td>
<td></td>
<td>itching</td>
<td>lived</td>
<td>emphysema (-)</td>
</tr>
</tbody>
</table>
| plasmin unit: 1.8 U/mg (TAMe), 3.0 U/mg (BAEe)

*++ --- medium, + --- slight - --- nothing

Fig. 2

Histamine remained 15.0 /ml in the deposit (Table 2. Fig. 1).
Plasmin, histone, and histone with plasmin were incubated for twenty-four hours in a 37°C incubator and after incubation each of them were injected intravenously to guinea pigs. The animals received histone caused anaphylactoid symptom and died with the emphysema of lung (Table 3 and Fig. 2). Histone with plasmin, however, caused the
itching alone and the lung emphysema did not develop (Table 3 and Fig. 3).

DISCUSSION

Protease activation has been observed in all type of tissue aggression, particularly in allergic response (3-6) and many papers (1, 2, 13) in the past have called attention to the possibility of explaining anaphylaxis as a resulting from the activation of proteolytic enzymes. Among them Rocha e Silva (3) advanced a protease theory to explain the histamine release in anaphylactic shock, because he (14) showed that anaphylactoid phenomenon was able to produce by injection of trypsin. Trypsin and papain released histamine from perfused guinea pig (15), rabbit blood cells (10) and platelets (16).

Most of the data favouring the enzymatic mechanism of histamine release have involved extracellular enzymes, mainly plasmin (fibrinolysin). The release histamine by plasmin has been very weak and inconsistent in actual experiments, even in high concentration is not able to release histamine (10, 11, 14). Consequently, some author (17,18) believes that protease activation is not the cause but the consequence of the allergic reaction.

Recently mammalian mast cells have been found to contain not only heparin but also histamine (19) and now mast cells are recognized as an important source of histamine.

With the knowledge of mast cell and the structure of mast granule a multiplicity of mechanisms of histamine release have been suggested
and the histamine release theories have to be modified from time to time as new experimental data emerge. These ranges from the total disruption of the mast cell to the quiet replacement of the histamine molecule by another amine without accompanying morphological change. Even though some experimental evidence can be found to support each of the various theories advanced, all of them seem to have serious shortcomings.

When histamine is released from the mast cell by a synthetic histamine liberators, antigen-antibody reaction, mechanical trauma, osmotic pressure and other, the mast cells in the tissue undergo profound morphological changes. The cell become degranulated and more or less lose their staining characteristics for basic dyes.

Among the histamine release theories various enzyme theories have been suggested (21,22). Archer (23) reported that trypsin with cysteine had disrupted the mast cells with the release of histamine. Papain and pepsin also were found to cause disruption of mast cell. In his experiments he collected the mast cells from the peritoneal cavity of rats. Högberg and Uvnäs (21) reported that trypsin and plasmin and other proteases were unable to disrupt mast cells in rat mesentery, even in high concentration did not.

Author (24) reported the significant amount of histamine release by trypsin and plasmin to rat mast cells collected from its peritoneal cavity. On the action of protease the mast cells in mesentery might be something different from that of collecting from peritoneal cavity.

Recently Archer (25) has described an endogenous histamine-liberator which may play a vital role in the release of histamine in the inflammatory process. He (23) has reported that extracts prepared from nucleated tissues not only release the histamine from the mast cells but disrupt them and suggested the chemical nature of the active principle in tissue extract appears to be an arginine containing polypeptide and it is possibly derived from histone.

On the classification of proteins histone belongs to the class of simple protein and it appear to contain relatively large proportions of arginine and lysine. It exists in the nuclei associated with the nucleic acids and it is basic protein.

Widal (27) reported that the white blood cell count is greatly decreased not only in anaphylactic shock, but also in acute allergic diseases. Immense number of those cells are destroyed in anaphylactic shock. Yamaguchi (27) reported that in patients with skin allergic diseases leukolysis was observed by adding the antigen in vitro and same phenomenon was observed by adding the plasmin and he suggested leukolysis mechanism might be caused by proteases, particularly plasmin. Tojo (28) observed that plasmin decreased leukocytes, chiefly of granular one, in vivo and in vitro and anti-plasmin (L-amino-capronic acid) could
suppress the decrease of leukocytes by adding the plasmin.

After destroying the cells by plasmin or antigen-antibody reaction histone in nuclei seems to be released from the cells.

On the experiments mentioned above histone caused the anaphylactoid reaction in guinea pigs and released the significant amount of histamine release from the mast cells but histone with plasmin incubated for twenty-four hours in a 37°C incubator did not cause the anaphylactoid reaction. The reason is not clear but plasmin might hydrolyze the histone.

Histamine is a base that is thought to be loosely linked to acid groups in the intracellular granules; and most synthetic histamine liberators are organic bases. There is some indirect evidence suggesting that histamine and heparin may exist in mast cell as a complex (29). Displacement theory (30) is that such substances liberate histamine by penetrating the cell and granular membranes and replace the histamine. This simple and hence attractive hypothesis might explain the histamine release produced experimentally by some organic bases. UVNÄS (22) doubts whether the histamine liberation observed clinically or experimentally even after minute doses of various substances can be explained simply on an ion exchange basis and suggested by his experimental results that a basic histamine-liberators release histamine from the mast cell, not by a mere neutralization of acidic heparin in a histamine-heparin complex, but by removing an inhibitor from a lecithinase situated at the mast cell membrane. The activated enzyme then brings about a lysis of the cell envelope, so permitting the intracellular histamine to escape.

Anyway histone caused anaphylactoid reaction in guinea pigs and

![Figure 4](image-url)

**Fig. 4**

1. Irritant
2. Activation of Protease
3. Lipoprotein
4. Histone
5. DNA
6. Histamine
7. Heparin Complex
8. Histamine
9. Mast Cell

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released histamine. Histone with plasmin, however, incubated in a 37°C incubator for twenty-four hours did not cause the anaphylactoid shock. Consequently, the author suggests that protease may attack the lipoprotein constituent of cell membrane and may destroy it. After destroying the cell histone from the nuclei may release easily. Released histone attacks the mast cells and releases histamine from them. Plasmin, however, attacks the histone and may hydrolyze it. Hydrolyzed histone may lose the character to attack the mast cell and may not release histamine, because histone with plasmin did not cause the anaphylactoid reaction in guinea pigs (Fig. 4). The reason why plasmin did not release the histamine from the tissue may ground in this point.

ACKNOWLEDGMENT.

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REFERENCES