On the Degranulation of Mesenteric Mast Cells Caused by Antihistamine in Albino Rats
—Effects of Various Dosages of Antihistamine—

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ABSTRACT

Degranulation of the mast cell has been reported by the injection of histamine liberators and other chemical agents. Chlorpheniramine maleate (1.2 mg./kg. and 0.3 mg./kg. comprising 1/74 and 1/290 of LD₉₀ respectively), which is an antihistamine agent, in physiological saline solution for intravenous injection and in Tyrode solution for intraperitoneal injection were given in single dose.

The mesenteric mast cells stained in Pugh solution, as applied by Lee (1968), were counted according to the classification of An (1964) in 4 types: the typical normal mast cell, the Grade I type of mast cell, the Grade II type of mast cell and the Grade III type of mast cell.

In the experimental rats given 1.2 mg./kg. of chlorpheniramine intravenously, more mesenteric mast cells were slightly degranulated than those cells of the rats given 0.3 mg./kg. of chlorpheniramine and the control rats.

In the experimental rats given 1.2 mg./kg. and 0.3 mg./kg. of chlorpheniramine intraperitoneally, more mesenteric mast cells were slightly degranulated than those cells of the control rats. However, in this intraperitoneal study the degree, or severity, of degranulation of the mesenteric mast cell was not in direct proportion to the dosage of this antihistamine. Consequently it is deduced that the experimental dosage of the antihistamine chlorpheniramine maleate, applied 1/74 and 1/290 of LD₉₀ caused an event degranulation of mesenteric mast cells of the albino rats associated with probable histamine liberation.

INTRODUCTION

The tissue mast cells, containing metachromatic or orthochromatic granules in the cytoplasm have been described in the literature as a source of heparin (Holmgren and Wilander, 1937), histamine (Riley and West, 1963), Hyaluronic acid (Asboe-Hansen, 1962), serotonin (Benditt et al, 1965), and certain mucopolysaccharides.

MacIntosh and Paton (1949) and Paton (1951) suggested that a number of different chemical compounds have the property of releasing histamine without producing gross structural change. These substances were called “histamine liberators”. Alam et al (1939), Rocha and Schild (1949), Mongar and Whelan (1953), Riley and West (1955) and Perry (1956) suggested that histamine of the mast cell was released by the administration of various chemical histamine liberators, such as curare, d-tubocurarine, compound 48/80 to experimental animals. At the same time, it has been demonstrated the mast cells were degranulated. Fawcett (1954, 1955) and Parratt and West (1957), and Klaus and Winkelmann (1959) demonstrated that injection of histamine liberators, such as compound 48/80, reserpine and stilbamidine caused the degranulation or disruption of the tissue mast cells and the release of histamine. Choi et al (1964)-
demonstrated when the serotonin liberator, reserpine, was injected locally into the rat mesentery, mast cells near the injecting site were severely degranulated.

Paff and Mergenthaler (1955) showed that protamine sulfate injected into the peritoneal cavity of a normal rat ruputures mesenteric mast cells within 15 to 30 minutes.

On the effect of antihistamine for degranulation and disruption of tissue mast cells, and histamine liberation, Pellerat and Murat (1946) reported that the concentration of histamine in blood was increased after the administration of various antihistamines. Riley (1953) believed that there is a direct effect by the histamine liberators on mast cells and it has been shown that these effects are prevented by pretreatment with an antihistamine drug. Arunlakshana (1953) and Tasaka (1957) reported that histamine was released from the tissue by antihistamines. Mota and Silva (1960) reported that when pieces of guinea-pig mesentery were incubated, in vitro with antihistamines in tyrode solution at 37°C, damage induced by antihistamines was very similar to that induced by octylamine by Mota (1959); characterized by diffusion of the metachromatic material and later dissolution of the cell. An (1964) suggested that antihistamine also causes degranulation and destruction of mast cells of the rat mesentery which might be correlated with the “histamine releasing” effects of antihistamine.

Based on the above reports, it is well known that certain antihistamines act as histamine liberators in mesentery mast cells. This study has been carried out to demonstrate the degranulation of mesenteric mast cells in the rat by the administration of antihistamine.

MATERIALS AND METHODS

The animals used in this experiment were 29 well developed mature albino rats (16 females and 4 males for the experimental groups and 10 males for the control groups) weighing approximately 200 grams each. These rats were divided into 4 experimental groups; the first given 1.2 mg./kg. of chlorpheniramine maleate in 1 ml. of physiological saline solution intravenously, the second 0.3 mg./kg. of chlorpheniramine maleate in 1 ml. of physiological saline solution intravenously, the third 1.2 mg./kg. of chlorpheniramine maleate in 10 ml. of Tyrode solution intraperitoneally and the fourth 0.3 mg./kg. of chlorpheniramine maleate in 10 ml. of Tyrode solution intraperitoneally. The two control groups were given the same volume of physiological saline solution respectively, but without the antihistamine. Injection routes were the same. 4 or 5 rats were used in each group.

All of the rats in the experimental groups were sacrificed by an occipital blow after 6 hours of the injection and in the control groups given intravenously and intraperitoneally were sacrificed after 2 hours and 4 hours of the injections. Then absolute methanol was directly infused into the peritoneal cavity through a small incision of the anterior median abdominal wall with the rat in the supine position for the fixation of the mesentery in situ in order to avoid artificial damage to the mesenteric mast cells during histological procedures. After 20 minutes of the fixation in situ a few pieces of the mesentery were carefully excised for the flat preparation and stained with Pugh solution containing toluidin blue for 3 minutes and then prepared for the permanent slide. The degrees of degranulation of mesenteric mast cells were classified in 4 Grades according to the method of An (1964); the typical normal mast cell, (Fig.1) the Grade I type (Fig.2) of the mast cell which was slightly degranulated, the Grade II type (Fig.3) of the mast cell which was moderately degranulated, and the Grade III type (Fig.4) of the mast cell which was disrupted.
RESULTS

1. The experimental groups given 1.2 mg./kg. and 0.3 mg./kg. of chlorpheniramine maleate intravenously: In the rats given 1.2 mg./kg. of chlorpheniramine maleate intravenously the mesenteric mast cells showed a slight degranulation of metachromatic cytoplasmic granules in 10.2±0.13%. In the rats given 0.3 mg./kg. of chlorpheniramine maleate intravenously, the Grade I type of the mast cell found in an incidence of 4.7±0.07%, was less numerous than those of the former group. In the degranulation of the mesenteric mast cell, the larger dose of antihistamine caused more secretion of metachromatic granules from the mast cells (Confer the Table).

2. The experimental groups given 1.2 mg./kg. and 0.3 mg./kg. of chlorpheniramine maleate intraperitoneally: In the experimental animals given 1.2 mg./kg. of antihistamine intraperitoneally, Grade I type of the mast cell occurred in 5.9±0.14% and in the animals given 0.3 mg./kg. they occurred in 9.6±0.15%. According to these results the secretion of metachromatic granules from the mast cells was not directly proportional to the large dosage of antihistamines given (Confer the Table).

Table: Result of degranulation of mesenteric mast cells injected intravenously and intraperitoneally

<table>
<thead>
<tr>
<th>Injected group</th>
<th>Quantities injected</th>
<th>Number of mast cells observed</th>
<th>Normal type</th>
<th>Grade I type</th>
<th>Grade II type</th>
<th>Grade III type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous injection</td>
<td>Chlorpheniramine maleate 1.2 mg./kg.</td>
<td>46,755</td>
<td>89.7±0.14</td>
<td>10.2±0.13</td>
<td>0.06±0.01</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine maleate 0.3 mg./kg.</td>
<td>85,131</td>
<td>95.1±0.07</td>
<td>4.7±0.07</td>
<td>0.2±0.01</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Normal saline solution 1 ml</td>
<td>32,261</td>
<td>97.8±0.08</td>
<td>2.2±0.08</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intraperitoneal injection</td>
<td>Chlorpheniramine maleate 1.2 mg./kg.</td>
<td>33,842</td>
<td>93.3±0.15</td>
<td>5.9±0.14</td>
<td>0.3±0.03</td>
<td>0.5±0.04</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine maleate 0.3 mg./kg.</td>
<td>36,531</td>
<td>89.5±0.16</td>
<td>9.6±0.15</td>
<td>0.7±0.04</td>
<td>0.2±0.02</td>
</tr>
<tr>
<td></td>
<td>Tyrode solution 10 ml</td>
<td>21,069</td>
<td>96.7±0.12</td>
<td>3.2±0.12</td>
<td>0.09±0.02</td>
<td>0</td>
</tr>
</tbody>
</table>

m(%)±standard error (%)
activity as compound 48/80, which was known as the most active histamine release.

Mota (1953, 1958, 1959), Mota and Vugman (1956), Humphrey and Mota (1959) demonstrated that the release of histamine in the animal experiment of anaphylaxis was established, and Mota and Dias Da Silva (1960) reported that concentration of antihistamines was required to release histamine from guineapig tissues. Mota and Ishii (1960) suggested that mast cell damage produced by the antigen in sensitized rat tissues was morphologically similar to that caused by compound 48/80, both agents causing extrusion of metachromatic granules. Histamine release was correlated with mast cell damage. Finally, they suggested that the histamine-releasing mechanism of the antigen-antibody reaction in anaphylaxis was very similar to that of compound 48/80. On the contrary, Copenhaver et al. (1953), Mongar and Schild (1956) believed that the histamine in anaphylaxis was only liberated from intact cells.

This study was undertaken in order to observe the effect of antihistamine on degranulation of the mesenteric mast cells of the albino rat after the administration of chlorpheniramine maleate intravenously and intraperitoneally. We found that the degranulation of the mesenteric mast cells occurred by the administration of antihistamine.

These results suggested that the mechanism blocking the terminal tissue receptors was associated with degranulation of the mast cells.

REFERENCES


LEGEND FOR FIGURES

Fig. 1. Intact (normal) mesenteric mast cells of the albino rat. Pugh stain 400X.

Fig. 2. Slight (Grade I) degranulated mesenteric mast cells of the albino rat. Pugh stain 400X.

Fig. 3. Moderate (Grade II) degranulated mesenteric mast cells of the albino rat. Pugh stain 400X.

Fig. 4. Disrupted (Grade III) mesenteric mast cells of the albino rat. Pugh stain 400X.