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Histamine Release from Isolated Rat Mast Cells by Adding Plasmin

Takuso YAMURA and Naoaki WATANABE*1

Department of Dermatology,
Nagasaki University School of Medicine,
Nagasaki, Japan

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The histamine release from isolated rat mast cells was accomplished by adding antigen, histamine liberators, and proteasse. The percentage of histamine release from the cells after adding the above mentioned agents is as follows : antigen, 41.5%; 48/80, 65.9; tween 20, 28.9; plasmin, 37.2; heated plasmin, 19.5; and trypsin, 42.8.

It has been widely accepted that mammalian mast cells contain, not only heparin but also histamine (1). In rats (2,3) and in mice (4) these cells may also contain 5-hydroxytryptamine. Histamine release induced by histamine liberators is usually associated with damage to mast cells (5,6,7,8,9). Mast cell alterations are also induced by the antigen-antibody reaction (9,10) and then histamine is released (9).

In mast cells, three types of proteases have been described; a trypsin-type enzyme (11), a leucine amino peptidase (12) and a chymotrypsin-like protease (13).

On the mechanism of histamine release in the allergic fields, various enzyme theories have been suggested. Among them protease theories have called much attention to the possibility of explaining histamine release as the resulting from the activation of proteolytic enzymes immediately after antigen-antibody reaction (15,16). However, the plasmin (fibrinolysin), serum protease has several weak points. One of them, the most important one is less histamine release for the same amount of protein broken down than either the anaphylactic or the anaphylactoid agents (16) and even in high concentration is not able to release histamine (17,18). Consequently, it is necessary to solve this problem by using the isolated mast cells of rats in vitro.

METHODS AND MATERIALS

Wistar rats of both sexes and weighing between 120 gm and 150gm were used. The animals were sensitized by injecting 1 ml of horse serum plus 1 ml of Haemophilus pertussis vaccine containing 20,000 million organisms intraperitoneally and were used three weeks later.

*1 矢村卓三・渡辺直昭

The mast cells were isolated by a method similar to that described by Glick, Bonting and Den Boer (19). 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 were allowed to reach room temperature after being stored 24 hours in a cold room. The interface was diffused by gentle stirring with a thin glass rod and then centrifuged for 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 was removed and replaced with Hanks' balanced salt solution.

Compound 48/80 (Burroughs Wellcome & Co.) was dissolved in phosphate buffer saline (pH 7.4) to a cencentration of 20γ/ml and tween 20 to a concentration of 1%.

Human plasmin was prepared by adding streptokinase to human serum and the titer was measured by method of Ungar and Mist (20).

Trypsin (Merck Co.) was dissolved in phosphate buffer saline (pH 7.4) to a concentration of 3.5 mg/ml.

Hanks' balanced salt solution containing mast cells was incubated for 5 minutes in a water-bath (37° C) after adding antigen (2 drops to 1 ml), histamine liberators (20γ/ml, same volume) and various protease (plasmin; 5120 U/ml, 0.5 ml to 1 ml, trypsin; 3.5 mg/ml, 0.5 ml to 1 ml) and centrifuged. After centrifuging both the supernate and the deposit were replaced by an equivalent amount of Hanks' balanced salt solution and the latter was heated in a boiling water-bath for 5 minutes. Histamine was measured by using usual bioassay on the guinea-pig ileum in Tyrode solution which contained 1γ/ml of atropine.

RESULTS

Histamine release caused by adding antigen, histamine liberators and various proteases on isolated mast cells is shown in Table 1. Among the results histamine release by plasmin and heated plasmin (O U/ml) are illustrated in Figure 1.

DISCUSSION

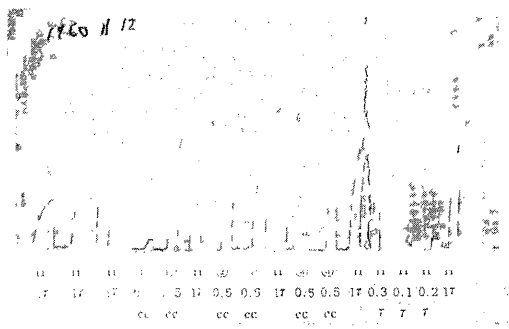
It has long been well established that histamine can be released from animal cellls under certain conditions. Riley and West (1) reported a close quantitative correlation of the histamine content of a tissue and the

Table 1

Agents	Concentration	Histamine in supernatant after addition	Histamine remaining in deposit	Total	Percentage histamine released
Antigen (Horse serum)	original (2 drops to 1 ml)	1.65 (γ)	2.33 (γ)	3.98(γ)	41.5 (%)
48/80	20γ/ml (same volume)	3.88	2.00	5.88	65.9
Tween 20	1 % (same volume)	0.62	1.52	2.14	28.9
Human plasmin	10 mg/ml (0.5ml to 1ml)	1.84	3.11	4.95	37.2
Heated human plasmin (60°C, 30 min.)	10mg/ml (0.5ml to 1ml)	0.64	2.63	3.27	19.5
Trypsin	3.5mg/ml (0.5ml to 1ml)	2.82	3.74	6.56	42.8

Plasmin units : 5120 U/ml, Heated plasmin: 0 U/ml

Fig. 1

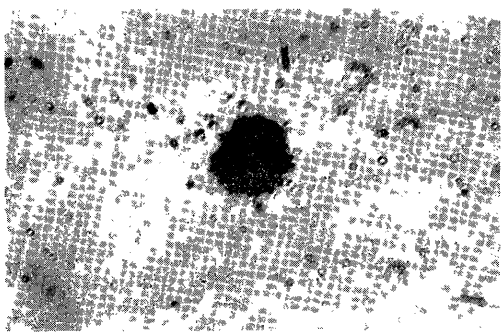


H--Histamine, ① --Histamine in supernatant after addition of saline (control), ①′ --Histamine remaining in deposit after addition of saline (control), ② --Histamine in supernatant after addition of plasmin, ②′ --Histamine remaining in deposit after addition of plasmin, ③ --Histamine in supernatant after addition of heated plasmin, ③′ --Histamine remaining in deposit after addition of heated plasmin

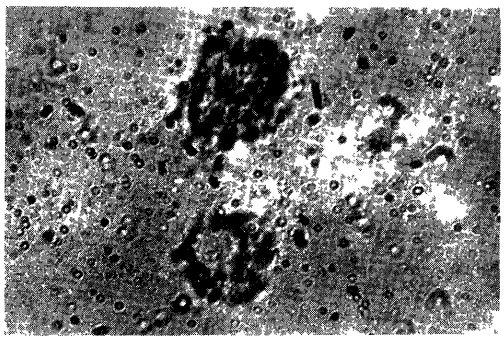
density of its mast cell population. The assumptjon of Riley and West that mast cells store histamine has been confirmed in various way and is now generally accepted.

When histamine is released from a tissue as the result either of an antigen-antibody reaction or the action of histamine libelators, the mast cells in the tissue undergo profound morphological changes. The cells become degranulated and more or less lose their staining charhcteristics for basic dyes (Fig. 2).

Fig. 2. A B



Mature Mast cell (Toluidine blue stain)



Mast cells immediately after antigen-antibody reaction (Toluidine bule stain)

It is interesting to emphasized here that the morphological difference between the mast cell damage, induced by the antigen-antibody reaction and by histamine releasers, might also reflect a difference in the mechanism of action of these agents on the mast cells, although both release histamine. This agrees with the fact that iodoacetate, phenol, calcium lack or heating to 45° C, although producing a partial or total inhibition of mast cell destruction by antigen in sensitized tissues, do not block the mast cell destruction by histamine releasers (octylamine and compound 48/80) (9).

At least three types of proteases have been described in mast cells ; a trypsin-type enzyme (11), a leucine amino peptidases (12) and a chymotrypsin-like protease (13).

The functional activities of these enzymes in vivo are not yet known. On attractive possibility is the liberation of secretory granule substance by the intracellular activation of the enzyme. Another possibility is that the enzyme is released from the cells along with heparin and histamine under various conditions. It is probable that histamine can be released in various ways, and it is thought by some that the most common mechanism is through the activation of a proteolytic enzyme.

Rocha e Silva (16), however, did not get any evidence of histamine release on many guinea pigs by perfusing the lung with Tyrode solution added with a potent fibrinolysin preparation. The effect of extracellular protease, fibrinolysin has been very weak and inconsistent in actual experiments and it was suggested that the release of histamine is associated with the intracellular activation of these enzymes rather than with extracellular proteases (14).

The histamine release from isolated rat mast cells after adding plasmin, however, was almost the same amount as when antigen was added, but heated plasmin (60° C, 30 min.) showed less histamine release. The reason plasmin did not release histamine when a guinea pig lung was perfused is not clear. It might interfere with some enzyme conta-

ined in the tissue or plasmin might hydrolyze some nucleus protein concerning with mechanism of histamine release (unpublished).

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