HISTAMINE RELEASE BY INORGANIC CATIONS FROM MAST CELL GRANULES ISOLATED BY DIFFERENT PROCEDURES

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Most of the histamine in mast cells is known to be stored in specific granules (1–3), but as for the mode by which histamine is bound within the granules, opinions are not in harmony. Thus, a hypothesis has been proposed that histamine is bound electrostatically to anionic sites of the heparin-protein complex of the granule constituents from which histamine is easily released by ionic exchange when the isolated granules are exposed to cations in the medium (4–7). However, many classical experiments have indicated that histamine in mast cell granules is not so susceptible to the releasing action of inorganic cations of the outer milieu (8–12). A possible reason for the discrepancy may be changes in the properties of mast cell granules during the isolation procedures. In the present paper, this assumption has been supported by the observation that there are considerable differences in the histamine release by inorganic cations from mast cell granules depending on the method of granule isolation.

MATERIALS AND METHODS

Mast cells in peritoneal washings using a physiological buffer solution—NaCl 154 mM, KCl 2.7 mM, CaCl₂ 0.9 mM and Sörensen phosphate buffer 6.7 mM (pH 7.4)—were collected from Wistar rats of both sexes weighing 150–250 g and isolated from other types of cells, as described previously (13). They were washed twice with 0.32 M sucrose and frozen and thawed three times in the same medium. The precipitate obtained after centrifugation at 7500 g for 10 min was used as mast cell granules. It retained about 60% of the total histamine content in washed mast cells. All the procedures for preparation of the granules were performed below 4°C in this and following experiments.

Homogenization of mast cells was used as another method to obtain the granules. Peritoneal mast cells not isolated from other types of cells were washed with 0.32 M sucrose containing 40 μM EDTA×2Na and 1 mM Tris-HCl buffer (pH 7.4), with or without 1% of bovine serum albumin, fraction V (BSA) (Armour Laboratories). Mast cells were homogenized in the same medium in a glass homogenizer with a Teflon pestle. Broken cells and nuclei were separated by centrifugation at 600 g for 10 min. The granule fraction was

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collected by centrifugation of the supernatant at 7500 g for 10 min. Suspension of this fraction in 0.32 M sucrose was smeared on a slide glass and gross moisture was removed by evaporation. Mast cell granules could be identified by the presence of a bright yellow fluorescence due to histamine, after the addition of a small drop of 1% of o-phthalaldehyde dissolved in p-xylene (14), under a fluorescence microscope. The granule fraction obtained in the presence or absence of BSA contained 17-27% of the total histamine content. Histamine content in the fraction of cell debris and nuclei was 31-44% of the total.

The fraction containing mast cell granules was also prepared from subcutaneous tissue of a young rat weighing about 100 g. Pieces of the tissue were scraped and homogenized in 20 ml of 0.25 M sucrose containing 40 µM EDTA•2Na and 1 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 600 g for 10 min, and the supernatant was layered over 30 ml of 0.32 M sucrose containing EDTA•2Na and Tris-HCl buffer and centrifuged at 1200 g for 10 min. After removing the upper layer, a 0.32 M sucrose layer was transferred into another centrifuge tube and spun down at 7500 g for 10 min. The resulting precipitate was used for histamine release experiments.

Mast cell granules were suspended in 2 ml each of the solutions to be tested for histamine-releasing effect, and incubated at 0°C or 37°C for 5-10 min in a polyethylene test tube. After centrifugation at 7500 g for 10 min in the cold, the supernatant was transferred into a glass test tube containing 0.2 ml of 1 N HCl. The precipitate was suspended in a solution consisting of 2 ml of 0.9% NaCl and 0.2 ml of 1 N HCl. After boiling, each sample was neutralized with 1 N NaOH and assayed for the histamine content on an isolated guinea-pig ileum.

RESULTS

Histamine release at 0°C from mast cell granules obtained by freezing and thawing is shown in Fig. 1. The amount of histamine released from the granules suspended in deionized water was about 30% of the total. This amount increased with an increase in the concentration of inorganic cations in the medium. Divalent cations were more potent than monovalent in this effect, and there was no difference among different cations of the same valency. Basic histamine releasers, such as compound 48/80 and sinomenine, released histamine from these granules to a degree comparable to the effects of these metal ions on the basis of molar concentration.

When mast cells were homogenized in an isotonic sucrose solution, the granules showed different properties depending on the presence or absence of BSA in the medium used for homogenization. As shown in Fig. 2, 10% of histamine was released from the granules obtained in the presence of BSA, during incubation in 0.32 M sucrose at 0°C. There was no significant increase in the histamine release from these granules with the addition of 1-10 mM of cations, irrespective of valency, or 1 µg/ml of compound 48/80 in the medium. The amount of histamine released from these granules suspended in physiological buffer solution, Tyrode solution, or isotonic NaCl solution, was 30-38% of the total. When these granules were suspended in deionized water, about 40% of histamine was released.
FIG. 1. Histamine release by inorganic cations, compound 48/80, and sinomenine in deionized water, from granules obtained from isolated rat peritoneal mast cells by freezing and thawing in isotonic sucrose solution. Incubation: at 0°C for 5 min.

FIG. 2. Histamine release from granules obtained by homogenizing isolated rat peritoneal mast cells. Medium used for homogenization contained sucrose 0.32M, EDTA 40 µM and Tris-HCl buffer 1 mM pH 7.4, with or without BSA (1%). Incubation: at 0°C for 10 min.

On the other hand, histamine release from the granules obtained in the absence of BSA was greatly increased by the addition of metal ions or compound 48/80 in an isotonic sucrose medium. The histamine-releasing effect was more prominent with divalent metal ions, as observed in the granules obtained by freezing and thawing. In all three types of solution—physiological buffer, Tyrode, and isotonic NaCl solutions—the histamine release from these granules was also remarkable, reaching about 80% of the total. In contrast to
these facts, in deionized water a smaller percentage of histamine was released from these granules than from the granules obtained in the presence of BSA. But, as shown in Fig. 3, when the granules obtained in the presence of BSA were suspended in deionized water at 0°C, the subsequent addition of NaCl (10 mM) released almost all histamine in the granules, whereas NaCl was virtually inactive in releasing histamine in the granules suspended in an isotonic sucrose solution.

The granule fraction obtained from subcutaneous tissue released about 70% of its histamine in deionized water at 0°C while only a small percentage was released in an isotonic sucrose solution. This granule fraction showed considerable resistance to the histamine-releasing effect of cations in the isotonic sucrose solution, as did the granules obtained by homogenizing mast cells in the presence of BSA.

DISCUSSION

It has been suggested that mast cell granules of the rat are mainly composed of a polysaccharide-protein complex formed by ionic linkage (4). An ionic binding between histamine and protein carboxyl groups of the heparin-protein complex has been proposed as the mode of storage of histamine in mast cell granules (7). This idea has been based on the release of histamine by inorganic cations from the granules obtained by lysis of rat mast cells in deionized water (5, 6). Results of the present experiments on mast cell granules obtained by freezing and thawing and by homogenization of these cells in an isotonic sucrose solution without the addition of BSA, appear to be consistent with the ionic binding of histamine in the granules, since histamine was easily released by cations added to the medium.

But, the histamine release by cations was greatly reduced in the granules obtained by homogenization of mast cells in the presence of BSA in the sucrose solution. A greater percentage of histamine was released in deionized water from the granules obtained by homogenization in the presence of BSA, than from the granules prepared similarly, but without BSA. Deionized water was also effective in releasing a major portion of histamine from mast cell granules obtained from subcutaneous tissue by homogenization, while
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cations, in a sucrose medium, were less effective. These results suggest that in the granules obtained by homogenizing mast cells in the presence of BSA a greater portion of histamine was present in a state protected from ionic exchange by extragranular cations, while histamine in granules obtained similarly but without BSA or by freezing and thawing was not so protected.

When the granules obtained in the presence of BSA were treated in deionized water, a marked increase in their sensitivity to the histamine-releasing effect of cations was observed. This indicates that a change had occurred through this treatment in the mode by which histamine is bound in the granules, and suggests that the original properties of the granules were better preserved in the presence of BSA during the isolation procedures.

Results of these experiments indicate that the structural normality of mast cell granules is of great importance in considering the mode of existence of histamine. In intact granules, anionic sites for the binding of histamine may not be accessible to exogenous cations, probably owing to the structural integrity of the granules. It is possible that in some isolated granules the ionic binding of histamine becomes labile as a result of injuries to the granules during isolation procedures.

Granules obtained from rat mast cells in the presence of BSA as well as those from rat subcutaneous tissue showed properties like those of the mast cell granules obtained from the liver of the dog (8–10, 12), the liver capsule of the sheep (10), and from the lung of the guinea pig (11). Therefore, the discrepancies found in the properties of different mast cell granules may not be solely due to species difference.

SUMMARY

In deionized water, inorganic cations were effective in releasing histamine from granules obtained from isolated rat peritoneal mast cells by freezing and thawing or by homogenization in isotonic sucrose solution. Granules obtained by homogenization of these mast cells in isotonic sucrose solution containing bovine serum albumin, and also granules obtained from rat subcutaneous tissue by homogenization in the sucrose solution without albumin, were however resistant to the histamine-releasing action of cations. Cations became effective in releasing histamine after pretreatment of these granules in deionized water. These observations indicate the occurrence of changes in the mode of existence of histamine in mast cell granules during isolation procedures.

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