HISTAMINE RELEASE FROM ISOLATED RAT MAST CELLS IN METAL ION-FREE MEDIUM*

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It is generally recognized that the histamine release from mast cells induced by a number of histamine releasers including compound 48/80 or by antigen-antibody reaction is accompanied by extracellular discharge of granules (degranulation), though this is not always the case (1–3). Uvnäs and Thon (4, 5) proposed a hypothesis that the mechanism of histamine release from mast cells by compound 48/80 consists of two consecutive steps: the one being the process of extracellular discharge of granules, and the other, that occurs subsequently, a process of the release of histamine from extracellular granules. They consider that the first process is energy-requiring but the second one is a non-enzymatic simple ion exchange which occurs in the extracellular fluid phase between granular histamine and inorganic cations. This hypothesis is largely based on the following observations:

1) Mast cells which have degranulated in vitro on exposure to compound 48/80 retain their ability to store granules and histamine, and also to discharge granules and histamine on a second exposure to compound 48/80. 2) In an isotonic sucrose solution, granules are discharged from mast cells by compound 48/80 but histamine is not released; if these granules are resuspended in a medium containing inorganic cations, histamine release does occur. The first observation seems to be substantiated by recent findings of Diamant et al. (6) and Tasaka et al. (7) that when compound 48/80 is repeatedly applied topically to the surface of a single mast cell by means of microelectrophoresis, degranulation occurs in response to each application. We have failed to wholly reconfirm the latter observation, however, since in our experiment, histamine release from mast cells was produced to a considerable degree by compound 48/80 in isotonic sucrose solution, although an additional histamine release could be observed from mast cells resuspended in NaCl solution.

The present paper offers evidence of such a release of histamine induced by compound 48/80 in isotonic sucrose solution free of metal ions and describes some observations made for elucidation of the mechanism of this phenomenon. A short account of this work has been published (8).

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MATERIALS AND METHODS

Isolation of mast cells. Male Wistar rats (200-300 g) were exsanguinated by severance of carotid arteries. Immediately after death, 10 ml of buffered physiological solution (154 mM NaCl, 2.7 mM KCl, 0.9 mM CaCl₂, M/15 Sörensen phosphate buffer (pH 7.2) 10% v/v) was injected into the peritoneal cavity. After gentle massage of the intestines through the abdominal wall for 90 sec, the abdominal fluid was collected with a pipette. The collected fluid was layered over 2 ml of 37% (w/v) buffered solution of bovine serum albumin and centrifuged at 110 g for 20 min at 0-4°C (9). The albumin solution was then removed using a pipette, diluted to 6 ml with the buffered solution and centrifuged at 250 g for 5 min. The sedimented mast cells were washed twice with cold 0.3 M sucrose solution, unbuffered or buffered with 1 mM Tris-HCl (pH 7.2), and suspended in the same sucrose solution (4°C). Deionized water was used for all solutions in the present experiments.

Experiments on degranulation and histamine release. Spontaneous degranulation and histamine release, which occurred in the absence of compound 48/80, were measured in the following way. 0.1 ml of mast cell suspension (containing 1-3 x 10⁵ cells) in unbuffered or buffered (pH 7.2) 0.3 M sucrose was kept at 4°C and added to 2.4 ml of the same sucrose solution which was maintained at various temperatures and then left standing for 5 min. 0.5 ml of the cell suspension was removed with a pipette and fixed by the addition of one drop of 4% formalin for morphological observation. The remaining 2 ml of the cell suspension served for determination of the percentage of histamine release. All the experiments in a sucrose solution were carried out in plastic tubes, since glass tube adsorbs free histamine on its surface in a cation-poor medium.

For testing degranulation and histamine release induced by compound 48/80, the isolated mast cells were washed with 0.3 M sucrose of pH 7.2, buffered with 1 mM Tris-HCl or in some experiments with 1 mM EDTA-Tris buffer, at 4°C and suspended in the same medium. 0.1 ml of this mast cell suspension (1-3 x 10⁵ cells) was added to the same medium of 2.3 ml cooled to 4°C and followed by addition of compound 48/80 making the total volume to 2.5 ml. The reaction was initiated by transferring this suspension to a water bath of 37°C immediately after addition of compound 48/80. After the reaction, 0.5 ml of the mixture was taken for the morphological examination of the mast cells and the remaining 2 ml for the measurement of the percentage of histamine release. In this experiment, the preservation time of mast cells in the sucrose solution before the addition of compound 48/80 was no longer than 60 min for the reason described below (Results, 2, a).

Morphological changes in the mast cells were examined with an invert-type phase-contrast microscope (Olympus PMB 480×). Mast cells with more than two granules on the outer surface were judged as degranulated. Percentage of degranulated cells was calculated from examination of 300 cells. For the determination of percentage of histamine release, the cell suspension was centrifuged at 0-4°C (3,000 g, 15 min), and contents of histamine in the supernatant (granules not contained) and precipitate were measured separately, as described before (10).

Experiment on time course of histamine release. The mast cell suspension in 0.9 ml
of 0.3 M sucrose (pH 7.2, with Tris-HCl) or buffered physiological solution (mentioned above) was kept at 4°C and added with 0.1 ml of compound 48/80 solution to make a final concentration of 0.5 μg/ml, then immediately transferred to a water bath of 37°C. After 2, 5, 10 or 30 min the reaction was terminated by addition of 5 ml of ice-cold respective medium, the cell suspension being simultaneously transferred to an ice bath. Percentage of histamine release in the cell suspension was determined as previously described.

Effect of metal ions on histamine release in sucrose medium. Mast cells (1-3 × 10⁵ cells) were suspended in 0.9 ml of 0.3 M sucrose (pH 7.2, with Tris-HCl) which contained either one of NaCl, KCl (1.5-9 mM), CaCl₂ or MgCl₂ (0.3-3 mM), at 4°C. This suspension was added with 0.1 ml of compound 48/80 (final concentration, 0.5 μg/ml), and then immediately transferred to a water bath of 37°C. After incubation for 5 min the reaction mixture was centrifuged at 3,000 g for 15 min and histamine release determined.

In another experiment, compound 48/80 (final concentration, 0.5 μg/ml) was added to 12 ml of 0.3 M sucrose solution (pH 7.2) containing mast cells (1-3 × 10⁶ cells) at 4°C and this reaction mixture immediately transferred to a water bath of 37°C. Five min after immersion, 2 ml of the reaction mixture was removed and centrifuged (3,000 g, 15 min,) for determination of histamine release during this period. The remaining 10 ml was incubated for another 10 min, then divided into five equal portions (2 ml) in centrifuge tubes, and centrifuged. The amount of histamine release during 10 min was estimated in the pooled supernatant. To examine the effect of NaCl on release of residual histamine contained in the sediments, the sediment in each tube was resuspended in 2 ml of NaCl solution or a mixture of NaCl and sucrose (pH adjusted to 7.0 with NaOH), incubated at 37°C, and histamine release was determined after 5 and 15 min.

Chemicals. Compound 48/80 was kindly supplied by Burroughs Wellcome Co., Inc., Tuckahoe, New York, and sinomenine hydrochloride by Shionogi Research Laboratories, Sagisu, Osaka. Toluidine Blue (Toluidine Blue-0) was the product of E. Merck AG, Darmstadt. Sucrose used throughout the present experiments was the specially prepared product, DGD-21, of Nakarai Chemicals, Ltd., Kyoto, and analytical data for Ca, Mg, K, and Na were 0.00002, 0.000005, 0.00003, and 0.00008 %, respectively. Other reagents were the purest grade products of the Wako Pure Chemical Industries, Ltd., Osaka.

RESULTS

1. Morphology of and spontaneous histamine release from mast cells in sucrose solution

Isolated rat mast cells suspended in an unbuffered 0.3 M sucrose solution at room temperature (20-25°C) did not retain normal configuration as in a balanced salt solution. Many of the cells had an irregular margin and a few to numerous granules were attached to the surface of the cell membrane. Extracellular discharge of these granules (degranulation) occurred gradually, the number increasing with time. Usually, degranulation was seen in a few limited portions of the cell membrane.

A similar abnormality in the morphology of mast cells was seen when the cells were suspended in mannose, galactose, lactose, maltose, or glucose solution of the same molar
concentration. Spontaneous degranulation also occurred in 0.137 M (isotonic) choline chloride solution.

a) Effect of tonicity of sucrose. Mast cells were incubated at 37°C for 5 min in sucrose solution of different concentrations. As shown in Fig. 1, in a sucrose solution of below 0.1 M concentration, mast cells underwent marked swelling and a part of the cells were lysed, but degranulated cells were hardly observed. In the range of 0.15-0.4 M concentrations 80-100% of the cell showed evidence of degranulation. Percentage of degranulated cells decreased in concentrations higher than 0.4 M, and the cells rather tended to shrink. Release of histamine from mast cells in a sucrose solution of concentration below 0.1 M was 50 to 80%, but decreased to 25% by increasing concentration to 0.15 M. This value was not altered by further increase in concentration of sucrose.

![Fig. 1. Effect of concentration of sucrose on spontaneous degranulation and histamine release from isolated rat peritoneal mast cells.](image)

b) Effect of temperature. Fig. 2 shows the effect of temperature on spontaneous degranulation and histamine release from mast cells incubated for 5 min in 0.3 M sucrose solution. Cell morphology was normal as that in buffered physiological solution at 37°C when the temperature of the medium was below 10°C. There was only a trifling release of histamine. When the medium was above 20°C, degranulation occurred in almost all the cells and histamine release rose to 25% although the latter did not increase further with temperature up to 50°C.

c) Effect of pH. Spontaneous degranulation and histamine release were determined with mast cells incubated at 37°C for 5 min in 0.3 M sucrose adjusted to various pH's with 1 mM phosphate or 1 mM Tris-HCl buffer. As shown in Fig. 3, the degranulation and histamine release were least in the range of pH 7 to 9 (more exactly, pH 7.2 to 8.2 for histamine release) in either of the buffers, and they increased on the acid side from pH 7. De-
**FIG. 2.** Effect of temperature on mast cells suspended in isotonic sucrose solution. Mast cells were incubated for 5 min in unbuffered 0.3M sucrose solution kept at indicated temperature. ○—○: spontaneous degranulation, ●—●: spontaneous histamine release. Each plot represents the mean value from 3 experiments.

**FIG. 3.** Effect of pH on spontaneous degranulation and histamine release from mast cells in isotonic sucrose solution. Mast cells suspended in 0.3 M sucrose solution containing 1 mM phosphate or Tris-HCl buffer were incubated for 5 min at 37°C. ○——○: degranulation in the solution with 1 mM phosphate buffer, ●—●: histamine release in the same solution. △——△: degranulation in the solution with 1 mM Tris-HCl buffer, ▲——▲: histamine release in the same solution. Each plot represents the mean from 3 experiments.

Granulation in unbuffered sucrose solution is probably due to the range of pH being 5.5 to 6.2.

d) Effect of the length of incubation period. Mast cells were incubated in unbuffered 0.3 M sucrose solution at 37°C for various periods of time. As shown in Fig. 4, about 70% of the cells showed evidence of degranulation after 2-min incubation, although the number of extruded granules increased with prolonged incubation, accompanied by irregularity of the cell surface. Some of the mast cells underwent lysis. Histamine release was about 20%.
FIG. 4. Time courses of spontaneous degranulation and histamine release from mast cells in isotonic sucrose solution. Mast cells in unbuffered 0.3 M sucrose solution were incubated at 37°C for various periods of time. ○—○: degranulated mast cells, ●—●: histamine release. Each plot represents the mean value from 3 experiments.

TABLE 1. Effect of metal ions on spontaneous degranulation and histamine release from mast cells in isotonic sucrose solution. Mast cells were suspended in unbuffered 0.3 M sucrose solution containing various inorganic salts and incubated for 5 min at 37°C.

| Salt   | Conc. (M) | Degranulation (%) | Histamine release (%) |
|--------|-----------|--------------------|-----------------------|
| None   | —         | 95.1±3.4           | 25.4±4.2              |
| NaCl   | 10⁻²      | 53.3±4.1           | 22.3±3.4              |
|        | 1.5×10⁻³  | 92.0±5.2           | 23.6±2.9              |
| KCl    | 10⁻²      | 45.6±2.8           | 20.6±2.4              |
|        | 1.5×10⁻³  | 87.5±6.3           | 24.7±3.2              |
| CaCl₂  | 5×10⁻⁴    | 24.3±4.2           | 13.4±3.4              |
|        | 10⁻⁵      | 33.2±5.3           | 18.3±5.2              |
| MgCl₂  | 5×10⁻⁴    | 21.2±3.5           | 11.2±3.3              |
|        | 10⁻⁵      | 28.4±3.3           | 17.4±4.6              |

Mean±S.E. of 4 experiments.

after 10-min incubation but increased more rapidly on continued incubation.

e) Effect of metal ions. Table 1 summarizes the effect of some metal ions on spontaneous degranulation and histamine release. Addition of 1.5×10⁻² M of NaCl or KCl had no effect on both spontaneous degranulation and histamine release. When either salt was added in 10⁻² M, the percentage of degranulation decreased by about 50%, though the decrease in histamine release was not significant. CaCl₂ and MgCl₂ apparently inhibited both degranulation and histamine release in a low concentration of 10⁻⁵ to 5×10⁻⁴ M.

2. Actions of histamine releasers on mast cells in isotonic sucrose solution

a) Effect of temperature of preservation of mast cells on the action of compound 48/80. The mast cells were suspended in 0.3 M sucrose buffered to pH 7.2 with 1 mM Tris-HCl,
FIG. 5. Effect of temperature of preservation of mast cells on the histamine release by compound 48/80.

Curve 1: Mast cells were preserved at 4° C in 0.3 M sucrose solution (pH 7.2), added with compound 48/80 (0.5 µg/ml) at indicated time of preservation, and immediately transferred to a water bath of 37°C. Histamine release was indicated for 5 min period of incubation after the addition of compound 48/80 (in all curves). Curve 2: Preserved at 37°C and added with compound 48/80 at indicated time of preservation.

Curve 3: Preserved at 4°C in the presence of compound 48/80 for indicated period of time and then transferred to a water bath of 37°C.

Curves 4 and 5 are control for curves 1 and 2, respectively, without addition of compound 48/80.

Each curve is based on duplicate experiments.

since it was shown in the preceding experiment that spontaneous degranulation and histamine release were least in the pH range of 7 to 9. When mast cells suspended in this sucrose solution were allowed to stand at 4°C, added with compound 48/80 (0.5 µg/ml), and immediately transferred to a water bath of 37°C, histamine release occurred to a degree of about 50% for 5 min. Curve 1 in Fig. 5 represents this histamine release in relation to the time of preservation of mast cells at 4°C before the addition of compound 48/80. This curve, together with curve 4 which represents the corresponding spontaneous release, shows that standing at 4°C had no marked effect on the reactivity of the cells up to 60 min, but standing beyond this limit gradually gave rise not only to a decrease in the reactivity but also to an increase in the spontaneous release of histamine. In contrast, curve 2 shows that reactivity of the cells to compound 48/80 is largely lost by standing at 37°C for 15 min. On the other hand, as shown in curve 3, when the mast cells are left to stand in the presence of compound 48/80 at 4°C for various length of time and then warmed to 37°C, the reactivity of the mast cells diminishes with longer time of contact with compound 48/80, and is almost annulled after 60 min.

b) Time course of degranulation and histamine release by compound 48/80. Fig. 6 shows time courses of degranulation and histamine release from mast cells by compound 48/80 in 0.3 M sucrose solution and also in the physiological salt solution, both buffered to pH 7.2. Mast cells were suspended in both media cooled to 4°C, compound 48/80 added, and im-
FIG. 6. Time courses of degranulation and histamine release by compound 48/80 in 0.3 M sucrose and in physiological salt solution. Mast cells were suspended in buffered 0.3 M sucrose (pH 7.2, with Tris-HCl) or physiological salt solution (pH 7.2, with phosphate buffer) kept at 4°C, added with compound 48/80 (0.5 \( \mu \)g/ml), and immediately warmed to 37°C. •—• : degranulation in 0.3 M sucrose solution, 0—0 : histamine release in the same solution. •—• : degranulation in physiological salt solution, 0—0 : histamine release in the same solution. Each curve is based on duplicate experiments. The spontaneous degranulation and histamine release has been deducted.

mediately transferred to a water bath of 37°C. In both media, about 90% cells of the total showed extrusion of granules within 5 min. Histamine release also reached maximum within 5 min in both media. In the sucrose solution it was somewhat lower (58%) than that in the salt solution (79%). Since it takes about one min for the medium temperature to rise from 4°C to 37°C, the reaction in both media must be termed equally in a quite rapid process.

c) Actions of sinomenine and toluidine blue. Effects on the mast cells of other basic histamine releasers, sinomenine (400 \( \mu \)g/ml) and toluidine blue (25 \( \mu \)g/ml) were examined in the buffered isotonic sucrose solution by the same method as in the experiment with compound 48/80 in curve I of Fig. 5. With sinomenine, percentage of histamine release was 48.3 ± 3.4 (S.E.) and that of degranulated cells 87.5 ± 2.2 (average of 5 experiments), while in the case of toluidine blue these were 54.5 ± 5.0 and 94.3 ± 1.3 (average of 5 experiments), respectively, indicating that these basic histamine releasers are also capable of releasing histamine in isotonic sucrose solution.

3. Effect of cations on histamine release by compound 48/80 in sucrose medium

a) Effect of metal ions. Three different experiments were performed. In the first experiment, metal ions, Na⁺, K⁺, Ca²⁺ or Mg²⁺ were present in cold 0.3 M sucrose solution (pH 7.2, with Tris-HCl) containing mast cells, before addition of compound 48/80. After addition of compound 48/80, the reaction mixture was warmed to 37°C. Results are shown in Table 2. Presence of these cations in concentrations used did not enhance histamine release by compound 48/80 in the sucrose solution, while higher concentration of these ions
TABLE 2. Effect of metal ions on histamine release from mast cells induced by compound 48/80. Inorganic salts were in the mast cell suspension (0.3 M sucrose, pH 7.2) before addition of compound 48/80 (0.5 µg/ml) at 4°C. Reaction mixture was incubated at 37°C for 5 min. The values are the means from 3 experiments.

| Salt    | Conc. (M) | Histamine release (%) |
|---------|-----------|-----------------------|
|         |           | without 48/80 | with 48/80 |
| None    |           | 4.5          | 50.2       |
| NaCl    | $9 \times 10^{-1}$ | 23.8       | 55.8       |
|         | $1.5 \times 10^{-2}$ | 5.4        | 52.5       |
| KCl     | $9 \times 10^{-3}$ | 24.8       | 56.5       |
|         | $1.5 \times 10^{-3}$ | 5.9        | 48.1       |
| CaCl$_2$| $3 \times 10^{-1}$ | 33.9       | 49.4       |
|         | $3 \times 10^{-4}$ | 8.6        | 58.9       |
| MgCl$_2$| $3 \times 10^{-3}$ | 11.7       | 56.5       |
|         | $3 \times 10^{-4}$ | 6.5        | 59.9       |

Fig. 7. Histamine release by NaCl from mast cells previously exposed to compound 48/80 in isotonic sucrose solution. Mast cells were suspended in 0.3 M sucrose (pH 7.2, with Tris-HCl) at 4°C, added with 48/80 (0.5 µg/ml), and then warmed to 37°C. After 15 min of incubation, mast cells were centrifuged (3,000 g, 15 min) and resuspended in NaCl or NaCl plus sucrose solution.

At 0 min, mast cell suspension was added with 48/80 and brought into a bath of 37°C; at arrow, mast cells were resuspended in 1, 0.15 M NaCl; 2, 0.3 M NaCl; 3, 0.15 M NaCl + 0.3 M sucrose; 4, 0.3 M sucrose. Each curve is based on duplicate experiments.

increased the spontaneous release of histamine.

In the second experiment, illustrated in Fig. 7, compound 48/80 was added to the mast cell suspension in cold 0.3 M sucrose (pH 7.2, with Tris-HCl), a part of histamine (about 50%) was then allowed to release at 37°C for 15 min (the release was actually completed within 5 min), and the suspension was centrifuged (3,000 g) at 1-4°C. The precipitate (containing granules) was resuspended in a solution of NaCl or a mixture of NaCl and
sucrose, and histamine release at 37°C in these media was examined. NaCl solution, either isotonic or hypertonic, produced an additional histamine release to a considerable degree but this action of NaCl was apparently inhibited by the presence of sucrose.

In the third experiment, sucrose solution (0.3 M) was buffered to pH 7.2 using 1 mM EDTA-Tris buffer to remove trivial quantities of metal ions contained in the medium of sucrose. The mast cells were suspended in this medium, cooled at 4°C, compound 48/80 added, and immediately transferred to water bath of 37°C. The reaction mixture was allowed to stand for only 1 min in the bath, since spontaneous release of histamine increased more rapidly in this medium than in the Tris-HCl buffered medium. In the presence of 0.5 μg/ml of compound 48/80, histamine release and degranulation were 65% and 97%, respectively, while spontaneous release of histamine was 18% (average of 3 experiments).

b) Concentration effect of compound 48/80. Histamine release from mast cells by compound 48/80 in 0.3 M sucrose (pH 7.2) reached a maximum 55% in 0.5 μg/ml concentration but did not increase with further increase in the concentration of compound 48/80 up to 100 μg/ml. This fact indicates that compound 48/80 itself is not utilized as a base for ion exchange with histamine in the granules.

c) Effect of Tris-HCl. Histamine release by compound 48/80 did not markedly vary by changing the concentration of Tris-HCl (pH 7.2) in 0.3 M sucrose in the range of 0.1 to 10 mM. Increasing concentration of Tris-HCl decreased the spontaneous histamine release in a sucrose solution (Table 3). Consequently, Tris-HCl itself is not utilized for the ion exchange of histamine in this medium.

| Tris-HCl (pH 7.2) (mM) | Histamine release (%) without 48/80 | with 48/80 |
|------------------------|-----------------------------------|-----------|
| 0                      | 18.2                              | 45.6      |
| 0.1                    | 12.5                              | 48.5      |
| 1                      | 5.3                               | 50.7      |
| 10                     | 4.5                               | 52.0      |

DISCUSSION

Mast cells isolated from rat peritoneal fluid, when suspended in isotonic sucrose solution at room temperature, did not retain the normal configuration as seen in physiologically balanced salt solution, and did not respond to compound 48/80. Johnson and Moran (12) observed that the mast cells isolated by sucrose density gradient centrifugation lost much of their histamine during the isolation procedure and also during the subsequent incubation in an isotonic salt solution, and these mast cells decreased the ability of releasing histamine
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in response to compound 48/80 or antigen. Cohn and Hirsch (13) reported that polymorphonuclear leukocytes from rabbit peritoneal fluid underwent spontaneous lysis in 0.34 M sucrose. Cytotoxic action of sucrose does not appear to be specific to mast cells. Since the same phenomenon has been observed with sugars other than sucrose, it is possible that such an action is shared by compounds having polyhydroxyl groups. Bivalent ions like Ca\(^{2+}\) and Mg\(^{2+}\) in a minute amount decreased spontaneous degranulation and histamine release of mast cells in an isotonic sucrose solution, suggesting the presence of some interaction between these ions and polyhydroxyl groups with respect to the stabilization of cell membrane although the mechanism is obscure at present.

For mast cells to retain normal morphology as well as normal histamine content in isotonic sucrose solution, it was necessary to adjust the pH of medium 7 to 9 and temperature to below 10°C. In an acidic medium below pH 7, degranulation and histamine release occurred spontaneously in mast cells. Even in a solution adjusted to pH 7.2 with Tris-HCl (1 mM), mast cells lost the ability to respond to compound 48/80 in a short while when warmed to 37°C. In the present experiments, histamine releaser was added at a low temperature (4°C) at which the mast cells retain their reactivity, and the reaction was initiated by immersing the mixture in a water bath of 37°C. With this procedure the histamine release of high reproducibility could be obtained in isotonic sucrose solution, since the reaction of mast cells to releasers was provoked before the cytotoxic effect of sucrose had time to develop. In experiments using such a technique, a considerable histamine release (50 to 58%) was induced in isotonic sucrose solution by 0.5 µg/ml of compound 48/80, with accompanying degranulation, although this release was less marked than the release in physiological salt solution which was about 80% at the same concentration of compound 48/80. In a similar experiment, 100 µg/ml sinomenine and 25 µg/ml toluidine blue released 48% and 54% of the total histamine of mast cells, respectively, in the sucrose solution, while corresponding values for the release in physiological salt solution have been reported as 75% (11) and 76% (2), respectively. These results indicate that the presence of sodium (or other cations) in the medium is not a requisite for histamine release by compound 48/80 and the other basic releasers. In the present experiment, histamine release reached a maximum in 0.5 µg/ml of compound 48/80 and further increase in the concentration of the compound failed to bring about a further increase in histamine release. This is to be expected since this concentration also produced a submaximal histamine release in physiological salt solution (9).

The percentage of histamine release, 50% or more, produced by 0.5 µg/ml of compound 48/80 in isotonic sucrose solution is much higher than the value, 10% or more, obtained by Then and Uvnäs (5) and further the release in their experiment increased progressively with the concentrations up to 4.0 µg/ml of compound 48/80.

Since the compound 48/80 induced histamine release in the sucrose solution buffered with EDTA-Tris, it is unlikely that a trivial quantity of metal ions still contaminated plays a role in the process of histamine release. Increasing concentration of Tris-HCl did not enhance the effect of compound 48/80. Therefore, it would be difficult to consider that
the basic substance in these cases had undergone exchange with histamine in the granules. Histamine release by compound 48/80 did not increase even when the sucrose solution contained inorganic salts such as NaCl or KCl (1.5-9 mM), or CaCl₂ or MgCl₂ (0.3-3 mM), so that the role of these inorganic ions on compound 48/80-induced histamine release in isotonic sucrose solution can not be great, if any. However, we can not definitely conclude that metal ions do not take part in the process of histamine release, since a possibility remains that cell membrane-bound metal ions — not removed by EDTA — play a role in the process of histamine release.

Histamine release from mast cells by compound 48/80 in isotonic sucrose solution was somewhat lower than in a balanced salt solution when examined by the method mentioned above. This could be due to the injurious effect of sucrose on mast cells even at a low temperature. This sucrose effect should be considered not only as an inhibition on the action of compound 48/80 on mast cells but also as an inhibitory effect on the process of histamine release from the granules which had been extruded or altered by compound 48/80. Favoring this view is the fact that the presence of sucrose decreases additional histamine release by NaCl from the mast cell precipitates (3,000 g) after application of compound 48/80. These results and the observation of Berthet and others (14) that the release of enzyme by NaCl from liver lysosomes was inhibited by sucrose, indicate that sucrose prevents the release of various substances from subcellular granules, although this mechanism remains unexplained.

Results obtained in the present experiments do not exclude the possibility that intracellular cations were utilized for ion exchange with granular histamine. However, since the concentration of granular histamine is as high as 0.4 M (5), it would be necessary for cations to be present in a considerable quantity and in a free form to induce remarkable histamine release. This is highly improbable.

The granules which have degranulated are usually without perigranular membrane (15). There are also observations which support the fact that histamine release occurs even without degranulation (1-3). Electron microscope studies by many workers revealed that in either of the cases the structure of granules is evidently altered from that of the normal granules (for references see Yamasaki et al. (2)). These "altered" granules (designation by Bloom and Haegermark (15)), as was discussed by Green (16), seem to signify that the internal structure of granule changed from the gel state to a swollen sol or state near it. These "altered" granules must have different mode of binding of histamine, and the majority of histamine has already been possibly liberated from the granules, and a part of it has remained in the granules due to their properties similar to those of ion-exchange resin or by simple adsorption on their surface. It is probable that the histamine easily released by Na⁺ is this kind of residual histamine, if not all. Our previous finding (17) that the resistance of granular histamine to the release by cations depends largely on the method of the preparation of the granules supports the above assumption.
SUMMARY

Mast cells were isolated from rat peritoneal fluid, washed, and suspended in isotonic sucrose solution. Spontaneous degranulation and histamine release were both minimal in the pH range of 7 to 9 and at temperatures lower than 10°C. Ca²⁺ and Mg²⁺ were effective in preventing spontaneous degranulation and histamine release. Reactivity of the mast cells to compound 48/80 was lost for the most part when the mast cells were preincubated in isotonic sucrose solution (pH 7.2, buffered with Tris-HCl) at 37°C for 15 min. When mast cells were suspended in isotonic sucrose solution (pH 7.2, with Tris-HCl or EDTA-Tris buffer) kept at 4°C, added with compound 48/80, and immediately transferred into a water bath of 37°C, a remarkable histamine release occurred, showing the same time course as in a physiological salt solution, although the release was less marked than in the latter solution. The presence of Na⁺, K⁺, Ca²⁺ or Mg²⁺ in the sucrose medium did not enhance this histamine release but when, after the effect of compound 48/80 in the sucrose solution, the mast cells (together with extruded granules) were resuspended in NaCl solution not containing sucrose, additional histamine release occurred, indicating that sucrose acts to inhibit the release of histamine from the granules. These observations explain why compound 48/80 was less effective in isotonic sucrose solution in releasing histamine from mast cells, but does not support the idea that inorganic cations in the extracellular fluid phase are indispensable in the mechanism of histamine release by compound 48/80 from isolated rat mast cells.

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