Naturally occurring aliphatic polyamines-induced histamine release from rat peritoneal mast cells

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Rat peritoneal mast cells were incubated with different concentrations of naturally occurring aliphatic polyamines, spermine and spermidine, at 0.1-10 mM and the amount of histamine release into the supernatant solutions was measured. The addition of each polyamine to the suspensions of the mast cells caused a histamine release in a dose-dependent manner. The effect of 10 mM spermine and spermidine was as much as that of 0.5 µg/ml compound 48/80. The histamine release from the cells incubated with each polyamine was rapid and the amount of histamine release into the supernatant solutions reached a maximum at 1 min with the incubations. 0.1 mM spermine, which in itself could not cause a significant histamine release, showed a tendency to enhance anti-IgE-induced histamine release from the mast cells.

Key words: histamine; mast cell; spermidine; spermine.

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Our recent investigations have indicated that increased metabolism of polyphosphoinositides may be an intrinsic part of the biochemical mechanisms that control mediator release from rat mast cells (5). At least 50% of total cellular polyphosphoinositide is present in the granules, which were shown to contain a highly active phosphatidylinositol kinase that catalyzes the formation of phosphatidylinositol 4-phosphate from endogenous phosphatidylinositol in the granule membranes (6). This enzyme in the granules has been demonstrated to be stimulated by naturally occurring aliphatic polyamines, such as spermine and spermidine (7).

Release of mediators from mast cells can be induced in a non-cytotoxic way by a number of agents including polybasic polypeptides, such as compound 48/80 and polymyxin B (8). The basic secretory pattern of mast cells has been shown to be the same whether the secretion is induced by these polyamines or an IgE-anti-IgE reaction (1). Aliphatic polyamines, such as spermidine and spermine, are naturally occurring cations and their ubiquitous distribution and the complexity of the mechanisms that regulate their intracellular concentrations suggest for these compounds important roles in normal cellular growth and differentiation (4, 9, 14). In the present report, possible effects of spermine and spermidine on rat mast cells were investigated by measuring the amount of histamine release into the supernatant solutions from the mast cells. Evidence will be presented that these naturally occurring aliphatic polyamines induce a histamine release from the cells.

MATERIAL AND METHODS

Material

Bovine serum albumin (BSA), toluidine blue, spermine tetrahydrochloride, spermidine trihydrochloride, compound 48/80, histamine dihydrochloride (Sigma Chemical Co., St. Louis, MO, USA); Percoll (Pharmacia Co., Uppsala,
Sweden); mouse monoclonal anti-rat IgE (anti-IgE) (Zymed Laboratories, Inc., South San Francisco, CA, USA); others (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Rat mast cell preparations

Rat mast cells mixed with leukocytes and peritoneal cells were obtained by peritoneal lavage of male Sprague-Dawley rats (250 to 300 g; Japan SLC, Inc., Shizuoka, Japan) in amounts of about $1 \times 10^6$ mast cells per rat as described previously (5, 6, 7). The lavage medium was a heparinized (10 U/ml) balanced salt solution (4.0 mM Na$_2$HPO$_4$, 2.7 mM KH$_2$PO$_4$, 150 mM NaCl, 2.7 mM KCl, 0.9 mM CaCl$_2$, pH 7.2) containing 0.046% (w/v) BSA (BSSA). The mast cells in this mixture were purified on a Percoll gradient (3) and were more than 90% pure by toluidine blue staining reactions.

Cell incubation conditions

Mast cells were washed twice with BSSA and resuspended. Mast cells, 1.5 to $2.0 \times 10^6$, in 0.9 ml of the medium were incubated at 37°C for 1 min and 0.1 ml of the polyamines solution in the concentration required was added and the mixture was further incubated for another minute. The reaction was stopped by the addition of 1 ml cold BSSA and immediate cooling in ice for 10 min. The incubation mixture was centrifuged at 70 g for 10 min at 4°C and the supernatant solutions were obtained for histamine assay. In some experiments, mast cells were incubated with anti-IgE for 45 min at 37°C in the presence and absence of spermine. Mast cells were also incubated with 0.5 µg/ml compound 48/80 under the standard conditions as control studies. Experiments were performed in duplicate.

Histamine assay

The amount of histamine in the supernatant solutions was measured with the fluorometric assay by the method of Shore et al (13). For the assay of total amount of histamine, HClO$_4$ was added to the mast cell suspensions at a final concentration of 4% (w/v). The mixture was centrifuged at 1000 g for 10 min at 4°C and the supernatant solutions were diluted two-fold with water for the assay. Experiments were performed in duplicate, and data on histamine release represent the means of duplicate values of net percent of total histamine. Differences between duplicates were less than 10%. In addition, all the major experimental observations have been studied in a number of experiments and were seen very consistently.

Lactic dehydrogenase assay

The release of lactic dehydrogenase into the supernatant solutions was measured by the method of Cabaud & Wroblewski (2).

Analysis of data

Unless otherwise stated, each point illustrated in each figure represents the mean and standard error of the mean (SEM) of three experiments. Significance was determined by employing the independent Student's t-test (two-tailed).

RESULTS

Effect of spermine and spermidine on histamine assay

Preliminary studies were conducted to check whether spermine and spermidine interfere with the fluorometric histamine determinations. 0.5 µg authentic histamine in the presence and absence of each polyamine was extracted under the standard procedures as described by Shore et al. (13) and was measured with the fluorometric assay. Fig.1 shows that spermine and spermidine at the concentrations used in the present study did not interfere with the fluorometric histamine assay.

Spermine and spermidine-induced histamine release from rat peritoneal mast cells

When the mast cells were incubated with different concentrations of spermine at 0.1–10 mM
for 1 min, histamine was released into the supernatant solutions in a dose-dependent manner and 1.0 mM and 10 mM spermine caused 16.3±2.8 (mean ± SEM) and 42.6±4.6% histamine release, respectively. The amount of histamine release induced by 10 mM spermine was significantly higher than that by 0.5 μg/ml com-

![Graph](image1.png)

**Fig. 1.** Effect of spermine and spermidine on fluorometric histamine determinations. 0.5 μg authentic histamine in the presence and absence of spermine (1) and spermidine (2) was extracted under standard procedures and was measured with the fluorometric assay. A: authentic histamine, B: authentic histamine and 0.1 mM polyamine, C: authentic histamine and 1 mM polyamine, D: authentic histamine and 10 mM polyamine.

![Graph](image2.png)

**Fig. 2.** Spermine-induced histamine release from rat peritoneal mast cells. Mast cells were incubated with different concentrations of spermine for 1 min. Each column represents the mean and standard error of the mean (SEM) of three experiments. A, B, C: 0.1, 1, 10 mM spermine, respectively; D: 0.5 μg/ml compound 48/80 as a control. * and **: statistical significance from the control at P<0.05 and P<0.01, respectively.

![Graph](image3.png)

**Fig. 3.** Time course for spermine-induced histamine release from rat peritoneal mast cells. Mast cells were incubated with 10 mM spermine and the amount of histamine release into the supernatant solutions was investigated serially. As shown in Fig. 3, the histamine release reached a maximum of 42.3±6.4% at 1 min with the incubation. Lactic dehydrogenase release from the mast cells incubated with 10 mM spermine into the supernatant solutions was <3% above compound 48/80 (30.1±2.0%) (P<0.05) and by 1.0 mM spermine (P<0.01) (Fig. 2). The cells were incubated with 10 mM spermine and the amount of histamine release into the supernatant solutions was investigated serially. As shown in Fig. 3, the histamine release reached a maximum of 42.3±6.4% at 1 min with the incubation. Lactic dehydrogenase release from the mast cells incubated with 10 mM spermine into the supernatant solutions was <3% above
Fig. 4. Spermidine-induced histamine release from rat peritoneal mast cells. Mast cells were incubated with different concentrations of spermidine for 1 min. Each column represents the SEM of three experiments. A, B, C: 0.1, 1, 10 mM spermidine, respectively; D: 0.5 μg/ml compound 48/80 as a control. * Statistical significance from the control at P<0.01.

Fig. 5. Time course for spermidine-induced histamine release from rat peritoneal mast cells. Mast cells were incubated with 10 mM spermidine for the indicated time. Each column represents the SEM of three experiments.

background. The mast cells incubated with different concentrations of spermidine at 0.1-10 mM for 1 min released histamine into the supernatant solutions in a dose-dependent manner. 1.0 mM and 10 mM spermidine caused 10.3±1.8 and 33.5±1.4% histamine release, respectively. The amount of histamine release induced by 10 mM spermidine was significantly higher than that by 1.0 mM spermidine (P<0.01), but there were no differences between the amount of histamine release and that by 0.5 μg/ml compound 48/80 (36.8±2.1%) (Fig. 4). The cells were incubated with 10 mM spermidine and the amount of histamine release into the supernatant solutions was investigated serially. As shown in Fig. 5, the histamine release reached a maximum of 38.5±3.1% at 1 min with the incubation. Lactic dehydrogenase release from the mast cells incubated with 10 mM spermidine into the supernatant solutions was <2.8% above background. Fig. 6 shows the comparison of the effects of spermine and spermidine on the histamine release. Namely, the mast cells were incubated with 1.0 mM spermine or spermidine for 1 min and the amount of histamine release induced by the polyamines was compared. 1.0 mM spermidine and spermine caused 12.9±1.2 and 14.8±2.5% histamine release, respectively, and there were no significant differences between the effects. The amount of histamine release induced by 1.0 mM of each polyamine was significantly less than that by 0.5 μg/ml compound 48/80 (32.6±2.1%) (P<0.01).

Effect of spermine on anti-IgE-induced histamine release from rat peritoneal mast cells

When the mast cells were incubated with anti-IgE (1/100 dilution) for 45 min, 11.8±2.5% histamine was released into the supernatant solutions as shown in Fig. 7. In the presence of 0.1 mM spermine, which in itself could not cause a significant histamine release, the mast cells stimulated by anti-IgE released 20.0±3.5% histamine, and 0.1 mM spermine showed a tendency to enhance the anti-IgE-induced histamine release from the mast cells (P<0.1).

DISCUSSION

Polyamines are ubiquitously distributed in animal cells, tissues and extracellular fluids and strongly associated with membranes and implicated in membrane function (12). Polyamines are polycations associated with nucleic acids and recognized as important regulators of cell functions, such as cell differentiation and proliferation (4, 9, 14). They are also shown to
interact with polyphosphoinositides (15) and cause an increase in the concentrations of polyphosphoinositides in the plasma membranes (16). Recent investigations from our laboratory indicated that naturally occurring aliphatic polyamines, such as spermine and spermidine, stimulated the phosphorylation of phosphatidylinositol in rat mast cell granules (7). Because \(^{32}\)PO\(_4\) incorporation into phosphatidylinositol 4-phosphate was increased during mediator release from rat mast cells (5), these findings led us to investigate possible effects of spermine and spermidine on rat mast cells by measuring the amount of histamine release into the supernatant solutions from the cells.

Preliminary studies showed that spermine and spermidine did not interfere with the fluorometric histamine assay. The addition of spermine and spermidine to the suspensions of rat mast cells caused a histamine release into the supernatant solutions in a dose-dependent manner. 10 mM spermine induced a significantly higher histamine release than that by 0.5 \(\mu\)g/ml compound 48/80, and 10 mM spermidine did as much as 0.5 \(\mu\)g/ml compound 48/80, which was used as a control polybasic histamine releaser in this study. The mast cells incubated with 10 mM spermine and spermidine were found to release histamine without cytotoxicity because the amount of lactic dehydrogenase release into the supernatant solutions was < 3% above background. The histamine release from the cells incubated with 10 mM spermine and spermidine was rapid and reached a maximum at 1 min with the incubations. When the amount of histamine release induced by 1.0 mM spermine and spermidine was compared, no significant differences were obtained.

The histamine release was seen only at relatively high concentrations of the polyamines. Polyamines, spermine and spermidine, are found in human blood in concentrations that vary from 2.3 to 3.8 \(\mu\)M (10), and increased concentrations of the polyamines in human body fluids have been documented in several physiological and pathological conditions, such as cystic fibrosis (11) and tumors (4). About the relevance of the concentrations of the polyamines used in this study, spermine and spermidine under the near concentrations have been shown to stimulate the phosphorylation of phosphatidylinositol in the plasma membranes (16).
and the mast cell granules (7). Even then, the concentrations of spermine and spermidine to induce histamine release are relatively high compared with the concentrations in body fluids, and it is therefore less likely that the polyamines play a role as direct histamine releasers. However, it would be more likely that the polyamines play an indirect role via suboptimal stimulation of the mast cell functions at much lower concentrations. Actually, 0.1 mM spermine, which in itself could not cause a significant histamine release, showed a tendency to enhance anti-IgE-induced histamine release from the mast cells.

Further studies are required for the conclusion because the mechanism behind the increase in histamine release by spermine and spermidine from rat mast cells could not be investigated directly in this study. One possible mechanism of action might be through the alterations in inositol phospholipid turnover in the cells. Because of the increased metabolism of phosphatidylinositol 4-phosphate in activated rat mast cells, which is thought to be produced enzymatically by phosphatidylinositol kinase (5), the stimulation of inositol lipid phosphorylation may have significant implications for the mechanisms of mediator release from rat mast cells. However, there is no obvious mechanism for producing rapid alterations in the kinase activity per se in activated mast cells as the enzyme is stimulated by Mg\(^{2+}\) or Mn\(^{2+}\) (6), which are not known to change dramatically in the cytosol during granule exocytosis from rat mast cells. Spermine and spermidine may induce the histamine release from the mast cells altering the kinase activity as natural polycations. Further, there may be important differences between rat peritoneal mast cells and human mast cells and it is impossible to extrapolate from rat to man until the investigations using human mast cells have been done.

REFERENCES


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