Synthetic Polycations, Polyethylenimines and Polyallylamines
Release Histamine from Rat Mast Cells

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Abstract—The effects of synthetic polycations, which induce liposomal membrane
fusion without inducing permeability changes, on histamine release from rat mast
cells were investigated. Polyethylenimines and polyallylamines with various
molecular weights released histamine from mast cells. Acetylated derivatives and
triethylentetramine did not release histamine or serotonin from the cells. The
histamine release induced by 10 μg/ml polyethylenimine with a molecular weight
of 600 was inhibited by 1 mM dibutyryl cyclic AMP, but not by 1 mM 8-bromo
cyclic GMP; 100 μM D-600, a calcium antagonist; or 30 μM W-7, a calmodulin
inhibitor. In the presence of polyethylenimines with molecular weights of 600,
1,200 and 1,800, no detectable release of cytosolic lactate dehydrogenase was
observed, indicating that histamine release induced by these polycations was not
due to their cytotoxicity. The potencies of these polymers in inducing histamine
release depended on their charges, but not on their degrees of polymerization. On
the other hand, the actions of polyethylenimine with a molecular weight of 10,000
and polyallylamines with molecular weights of 3,000–4,000 and 10,000 in releasing
lactate dehydrogenase were somewhat cytotoxic. These polycations did not
induce serotonin release from rat platelets, suggesting that platelets have no coupling
system of signal transduction by these polycations. Thus polycations seemed to
interact with the mast cell membrane to induce histamine release, and the potencies
of these polycations on mast cells seemed to differ from those of their effects on
liposomes, which were examined previously.

Various polycations, compound 48/80, polylysine, polymixin B, mastoparan, mellitin,
substance P, somatostatin, adrenocorticotropic hormone and protamine were found to
release histamine (1–8). It is noteworthy that mastoparan and mellitin, unlike compound
48/80, also induced serotonin release from platelets (9, 10). Moreover, some polycations
facilitate membrane fusion, when a component of the membrane is negatively charged
(11).

Recently, some polycations PEIs and PAAs, were found to induce liposomal mem-
brane fusion without inducing permeability changes (12). These polymers are essentially
harmless to humans and are used as additives in various pulps and papers (FDA PART 175–
105, –320, 176–351) and for various other industrial purposes. These polycations caused
aggregation of sonicated vesicles composed of egg yolk phosphatidylcholine and bovine brain phosphatidylserine (molar ratio, 4:1), and they also induced intermixing of liposomal membranes. The acetylated derivatives, however, did not cause aggregation or fusion (13). In this work, we examined the effects of these polycations on rat mast cells and platelets.

Materials and Methods

Preparation of rat mast cells and platelets: Mast cells and platelets were obtained from male Sprague-Dawley rats weighing 300–350 g. Mast cells from the peritoneal cavity were purified by using Ficoll 400 (Pharmacia) as described previously (14). The purity of the mast cells in the final preparations was more than 90%. [14C]Serotonin was incorporated into mast cells by the method of Stechschulte and Austen (15) with a minor modification. Briefly, 1 ml of suspension of purified mast cells (10^6 cells/ml) in Hepes-buffered Tyrode solution containing 0.03% bovine serum albumin, pH 7.4, was incubated with 1 μCi of [14C]serotonin for 1 hr at 37°C, and then the cells were washed three times.

Blood was collected from the carotid artery, with 1/7 volume of acid-citrate-dextrose (65 mM citric acid and 85 mM sodium citrate in 2% glucose) as anticoagulant, and platelet-rich plasma was prepared as described by Pinckard et al. (16). The platelets were labeled by incubating 5 ml of platelet-rich plasma with [14C]serotonin (2 μCi/tube) at 22°C for 30 min. After incubation, the mixture was layered onto a 2 ml cushion of Ficoll-Paque (Pharmacia), and the platelets were precipitated by centrifugation at 750×g for 20 min. The fraction containing platelets was suspended in Tyrode-gelation solution, pH 6.5, and layered onto another 2 ml of Ficoll-Paque. The purified platelets were resuspended in Tris-Tyrode solution, pH 7.2, at a final concentration of 11.1±0.67×10^8 platelets/ml.

Assays of histamine and serotonin release from rat mast cells: A sample of 1 ml of cell suspension (2×10^4 cells) in Hepes-buffered Tyrode solution was preincubated with W-7, dibutyryl cyclic AMP or 8-bromo cyclic GMP for 10 min or with D-600 for 30 sec at 37°C and then incubated with polycations for 10 min. Ice-cold Hepes-buffered Tyrode solution (1.8 ml for the histamine assay and 0.6 ml for the serotonin assay) was added to terminate the reaction, and the mixture was centrifuged at 2,100×g for 10 min at 4°C. Histamine in the supernatant was determined by the fluorometric assay of Shore et al. (17). As triethylentetramine interferes with the determination of histamine, we measured the release of radioactivity from mast cells labeled with [14C]-serotonin (12,039±1,546 dpm/2×10^4 cells/ml, n=8). The radioactivity in aliquots of the supernatant (0.8 ml) was determined in a scintillation spectrometer (Packard Tri-Carb 4530). Spontaneous release in the absence of polycations was 1,219±156 dpm/2×10^4 cells/ml (n=8). Histamine release and serotonin release are expressed as percentages of their total cell contents. Spontaneous release of histamine was less than 10%. Values for histamine and serotonin release are given as means±S.E. for three or four replicate experiments on different samples of pooled cells. ED50 means the concentrations of polycations required to produce 50% release of the control release at their maximal effective doses. The inhibitory effects of drugs were calculated by the following equation:

\[
\% \text{ inhibition} = 100 - \frac{[\text{histamine release with drug}]}{[\text{histamine release without drug}]} \times 100
\]

Moreover, mast cells were incubated with polycations at 37°C for 3 min, and then examined by phase-contrast microscopy (Olympus, Japan), and the cell shape and formation of aggregates were recorded in photographs at room temperature.

Assay of serotonin release from rat platelets: Aliquots of platelet suspension (3.47±0.19×10^4 dpm/5.56±0.34×10^8 platelets/500 μl, n=6) in Tris-Tyrode solution, pH 7.2, with 1 mM CaCl2 were incubated with 10 μl of thrombin or polycation at 22°C. After 4 min, the reaction was terminated by addition of 1.5 M formaldehyde (10 μl) and cooled in ice. The cells were removed by centrifugation at 12,000×g for 1 min at 4°C, and aliquots of the supernatant (400 μl) were taken for measurement of released [14C]serotonin. The release of serotonin was determined by scintillation
spectroscopy and expressed as a percentage of the release from the same volume of platelets after addition of 10 μl of 10% (w/v) Triton X-100.

Assay of lactate dehydrogenase release: Suspensions of purified mast cells were incubated with polycation under the same conditions as for assay of histamine release. After incubation with a polycation for 10 min, the incubation mixture was centrifuged, the supernatant was decanted, and the precipitate was suspended in 100 μl of Hepes-buffered Tyrode solution containing 0.1% (w/v) Triton X-100. The lactate dehydrogenase content of the resulting lysate (50 μl) was measured with a lactate dehydrogenase assay kit (LDH UV-Test Wako) from Wako Pure Chemicals (Osaka, Japan). The content of lactate dehydrogenase in the supernatant could not be determined, because polycations were found to interfere with the assay. However, they caused no interference with its assay in the pellet.

Determination of cytosolic calcium: Cytosolic calcium was measured with the fluorescent indicator dye fura-2 (18). For loading of fura-2, the cell suspension of purified mast cells was incubated with fura-2 acetoxymethylester (membrane permeable ester, 3.3 μM/10^6 cells) at 37°C for 45 min. The cells then were washed three times with centrifugation (400 x g, 5 min, 4°C) and re-suspended in Hepes-buffered Tyrode solution at a concentration of 10^6 cells/ml. Fura-2 acetoxymethylester is converted to fura-2 by esterase in the cytosol. The cell suspension (1 ml) was placed in a quartz cuvette in a temperature controlled (37°C) spectrophotofluorimeter (CAF-100, Japan Spectroscopic Co., Tokyo), in which the suspension was stirred gently with a magnetic stirrer. Fluorescence of fura-2 from mast cells was excited alternately by light at 340 and 380 nm, and the relative intensity of fluorescence at 500 nm was measured. A total of three signals (two fluorescence intensities and the F_{340}/F_{380} ratio) were recorded on magnetic tape. Increase of this ratio represented the increase in the intracellular concentration of free calcium.

Statistical analyses: Statistical significance was evaluated by the unpaired Student’s t-test, and a P value of 0.05 was taken as the upper limit of significance.

Chemicals: PEIs and PAAs (Fig. 1) were gifts from Nippon Shokubai Co. (Osaka) and Nitto Boseki Co. (Tokyo), respectively. PEI was composed of 25% primary, 50% secondary and 25% tertiary amines. Acetylated derivatives were synthesized as described previously (12). The molecular weights of these polymers were determined by polystyrene gel filtration, and several polymers (PEI_6, PEI_18, PEI_100, PEI_300, PAA_30-40 and PAA_100) were purified further by ultrafiltration. PEIs and PAAs are stable in aqueous solution, and their histamine-releasing potencies did not decrease during storage in aqueous solution for more than three months (data not shown). Concanavalin A, compound 48/80, 8-bromo cyclic GMP, and dibutyryl cyclic AMP were purchased from Sigma Chemical Co. (St. Louis, MO). Hepes and fura-2 acetoxymethylester were purchased from Dojindo Laboratories (Kumamoto, Japan). W-7 [N-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide] and D-600 (methoxy verapamil) were from Seikagaku Kogyo (Tokyo) and Knoll A.G. (F.R.G.), respectively. [1^4C]Serotonin ([1^4C]-5-hydroxytryptamine creatinine sulfate) and thrombin were purchased from Amersham International (Bucks, U.K.) and Mochida Pharmaceutical Co. (Tokyo), respectively. All other chemicals were of reagent grade. The following media were used: Hepes-buffered Tyrode solution, consisting of 137 mM NaCl, 2.7 mM KCl, 12 mM Hepes, 1 mM MgCl_2, and other media were prepared as previously described.

Fig. 1. Structures of polymers. A, polyethylenimine; B, polyallylamine; C, triethylentetramine.
mM CaCl$_2$, 5.6 mM dextrose and 0.03% bovine serum albumin, pH 7.4; Ca-free Hepes-buffered Tyrode solution of the same composition, but without 1 mM CaCl$_2$; Tyrode gelatin solution, consisting of 137 mM NaCl, 2.7 mM KCl, 12.1 mM NaHCO$_3$, 1 mM MgCl$_2$, 5.6 mM dextrose, 0.1 mM EGTA and 2.5 g/l gelatin, pH 6.5, and Tris-Tyrode solution, consisting of 137 mM NaCl, 2.7 mM KCl, 10 mM Tris, 1 mM MgCl$_2$, 5.6 mM dextrose and 2.5 g/l bovine serum albumin, pH 7.2.

**Results**

**Effects of polycations on histamine release from rat mast cells:** PEI$_6$, PEI$_{12}$, PEI$_{18}$, PEI$_{100}$ and PEI$_{300}$ caused dose-dependent release of histamine from cells incubated at 37°C for 10 min in the presence and absence of 1 mM CaCl$_2$ (Fig. 2). This Ca-independent histamine release induced by PEIs was not inhibited by 0.2 mM EGTA. An analogue of PEI$_{10}$ with a -OH group (PEI$_{10}$-OH, Epomin® *SP-110) also induced histamine release. Under our conditions, compound 48/80 at 1 μg/ml induced the release of 73.6±0.8% (n=7) and 61.1±2.4% (n=6) of the total cell content of histamine in the presence and absence of 1 mM calcium, respectively; its ED50 values in the presence and absence of 1 mM calcium were 0.07 and 0.12 μg/ml, respectively. In the presence or absence of calcium, the low molecular weight analogue triethylentetramine did not release [$^{14}$C]serotonin, whereas PEI$_6$ released radioactivity (Fig. 3), suggesting that small molecules of PEIs are inactive.

Poliallylamines, PAA$_{30-40}$ and PAA$_{100}$, released histamine from mast cells in the presence of 1 mM calcium. Unlike in the case of PEIs, the histamine releases induced by PAA$_s$ in the absence of calcium were about one-third of those in the presence of calcium, suggesting that part of the histamine release induced by PAA$_s$ is calcium-dependent. Addition of calcium shortly after pretreatment with PAA$_{100}$ in the absence of calcium resulted in histamine release. The histamine

![Fig. 2](image-url)  
**Fig. 2.** Histamine release induced by various polycations in the presence and absence of extracellular calcium. Purified mast cells were preincubated for 10 min and then a polycation was added. Histamine release was determined after incubation with the polycation for 10 min. Values are means±S.E. for 3–4 replicate experiments. When no vertical bar is shown, the S.E. was less than 3%. ○, release in Ca-free medium. ●, release in the presence of 1 mM calcium. A, PEI$_6$; B, PEI$_{12}$; C, PEI$_{18}$; D, PEI$_{100}$; E, PEI$_{300}$; F, PEI$_{10}$-OH; G, PAA$_{30-40}$; H, PAA$_{100}$.

![Fig. 3](image-url)  
**Fig. 3.** [$^{14}$C]Serotonin release induced by PEI$_6$ and triethylentetramine. Purified mast cells radiolabeled by [$^{14}$C]serotonin were preincubated for 10 min and incubated with PEI$_6$ or triethylentetramine for 10 min in the presence of 1 mM CaCl$_2$. Radioactivity in the supernatant was measured in a scintillation spectrometer. Values are means±S.E. for 3–4 replicate experiments. When no vertical bar is shown, the S.E. was less than 2%. ●, release induced by PEI$_6$; ○, release induced by triethylentetramine.
release induced by PAA100 (10μg/ml) decreased rapidly with time: the half-time of decay was about 2 min, and after 5 min, no significant calcium-dependent histamine release was detectable.

To compare the effects of polycations on histamine release with their effects on fusion of liposomal membranes in the presence and absence of calcium, we examined the shape of mast cells. PAA100 induced aggregation and fusion of the cells. On the other hand, PEIs did not induce appreciable aggregation of the mast cells. Triethylentetramine did not induce aggregation or fusion.

Release of lactate dehydrogenase from mast cells: The activities of the polycations for releasing lactate dehydrogenase did not correspond to their activities for releasing histamine (Table 1). PEI6, PEI12, PEI18 and PEI10 -OH did not release a significant amount of lactate dehydrogenase, whereas PEI100, PEI300, PAA30-40 and PAA100 released this enzyme. Triethylentetramine did not release lactate dehydrogenase: the % release with triethylentetramine (10 μg/ml) at 37°C for 10 min was -3.40±2.40% (n=3).

Effects of polycations on serotonin release from rat platelets: Table 2 shows the releases

| Polycation | in the absence of calcium | in the presence of calcium |
|------------|---------------------------|---------------------------|
|            | % Release (n)             | % Release (n)             |
| Spontaneous release | 0.00 (4) | 0.00 (4) |
| PEI6       | 8.2± 3.0 (4)            | 6.7± 9.4 (4)            |
| PEI12      | 5.9± 3.1 (4)            | 9.2±13.2 (4)            |
| PEI18      | 3.7± 1.6 (3)            | -3.8±17.4 (4)           |
| PEI100     | 24.0± 5.1 (3)           | 30.8± 6.1 (5)           |
| PEI300     | 30.9±10.4 (4)           | 32.1± 9.0 (5)           |
| PEI10-OH   | 9.0± 2.5 (3)            | 4.9± 6.4 (5)            |
| PAA30-40   | 64.0± 4.7 (3)           | 50.8± 8.1 (5)           |
| PAA100     | 77.1± 1.6 (3)           | 60.1± 9.4 (5)           |

Purified mast cells were incubated with various polycations (10 μg/ml) at 37°C for 10 min and then centrifuged. The lactate dehydrogenase activity in the cell pellet was then determined.

| Polycation | μg/ml | Enhancement of % release of [14C]serotonin by polycation |
|------------|-------|--------------------------------------------------------|
| PEI6       | 10    | 1.47±0.79                                               |
| PEI12      | 10    | 0.12±0.27                                               |
| PEI18      | 10    | 1.22±0.70                                               |
| PEI100     | 10    | 4.10±1.08                                               |
| PEI300     | 10    | 0.98±0.21                                               |
| PEI10-OH   | 10    | 0.55±0.13                                               |
| PAA30-40   | 10    | 0.90±0.35                                               |
| PAA100     | 10    | 3.88±1.33                                               |
| Compound 48/80 | 1 | -0.05±0.35                                             |
| Thrombin   | 1 U/ml | 57.8±5.43                                             |

Labeled rat platelets were incubated with various polycations or thrombin for 4 min at 22°C. Values were corrected for spontaneous release (9.13±1.20% of total serotonin content, n=12).
of serotonin induced by polycations. Treatment with 10 µg/ml of polycations did not increase the release of [14C]serotonin. Compound 48/80 at 1 µg/ml also did not induce release of radioactivity. On the other hand, 1 U/ml of thrombin effectively released [14C]serotonin. Incubation of platelets with polycations at 37°C for 4 min or at 22°C for 10 min also did not result in release of [14C]serotonin (data not shown).

Effects of the acetylated derivatives of PEI6 and PEI18: PEI6 was studied further because it was the most effective PEI for inducing histamine release, as described above. As shown in Fig. 4A, elimination of the positive charge on the imine nitrogen decreased the histamine-releasing potency of PEI6. Like the fusogenic activity of PEI6, its histamine-releasing activity in the presence and absence of calcium was decreased by its acetylation. The acetylated derivative of PEI18 also had no histamine-releasing activity (Fig. 4B). Moreover, these acetylated derivatives did not inhibit histamine release induced by compound 48/80, suggesting that they were not able to block the binding of compound 48/80 to mast cells. These derivatives did not induce aggregation or fusion, and they did not release lactate dehydrogenase from mast cells: % release of lactate dehydrogenase with 10 µg/ml of acetyl PEI6 and acetyl PEI18 at 37°C for 10 min were -1.23±1.93 and -1.27±4.08%.

Fig. 4. Comparison of the effects of acetylated derivatives of PEIs with those of the parent molecules. Histamine release from mast cells was determined after incubation with the polymer for 10 min in the presence of 1 mM calcium. Values are means±S.E. for 3 replicate experiments. When no vertical bar is shown, the S.E. was less than 2%. •, parent molecule; O, acetylated derivative. A, PEI6; B, PEI18.

Fig. 5. Time courses of histamine release induced by PEI6, PEI18 and PAA100. Values are means±S.E. for 3 replicate experiments. When no vertical bar is shown, the S.E. was less than 2%. Open symbols indicate spontaneous histamine release. PEIs and PAA100 were added at 10 µg/ml. A, release induced by PEI6; B, release induced by PEI18; C, release induced by PAA100.
Table 3. Effects of D-600 and W-7 on histamine release induced by various polycations

| Drug | % Inhibition of Drugs on Histamine Release Induced by |
|------|--------------------------------------------------|
|      | PEI6 ($\mu M$) | PEI18 ($\mu M$) | PAA100 ($\mu M$) | Compound 48/80 ($\mu M$) |
| D-600 | 10 | -1.50±1.93 | 3.45±8.06 | 4.22±3.42 | 0.65±2.80 |
|       | 30 | 1.22±2.35 | 1.50±5.99 | 3.35±1.06 | -0.32±1.94 |
|       | 100 | -2.97±2.73 | 1.72±1.81 | 12.45±5.08 | -6.40±2.90 |
| W-7   | 5 | 3.63±2.54 | -16.70±9.55 | 1.62±3.05 | 2.08±1.80 |
|       | 10 | 1.10±3.16 | -15.60±9.54 | -6.98±5.83 | -0.12±2.75 |
|       | 30 | 2.53±4.18 | -6.44±8.28 | -11.57±11.34 | 7.43±2.82 |

Purified mast cells (2×10^4 cells/ml) were preincubated with D-600 for 30 sec, and with W-7 for 10 min, then incubated with PEI6 (10 µg/ml), PEI18 (10 µg/ml), PAA100 (10 µg/ml) or compound 48/80 (1 µg/ml) for 10 min (n=6). % Inhibition was calculated as described in Materials and Methods.

respectively (n=3).

Time course of histamine release by polycations: The histamine release induced by PEI6 (10 µg/ml) amounted to 44.3±2.7% (n=6) of the total histamine content after 1 min and to 61.4±4.5% (n=6) after 10 min (Fig. 5). The time courses of the effects of PEI18 and PAA100 were compared with that of PEI6. Half maximum of histamine release was achieved 17, 17 and 38 sec after the additions of PEI6, PEI18 and PAA100, respectively. These times were longer than those with 1 µg/ml compound 48/80, because after incubation with compound 48/80 for 30 sec, the cells released 76.6±2.5% of the total histamine (n=4), and the half maximum of histamine release by compound 48/80 was achieved in less than 15 sec.

Roles of extracellular and intracellular calcium in histamine release induced by polycations: The effects of the calcium antagonist D-600 and the calmodulin inhibitor W-7 on histamine release in the presence of 1 mM calcium were examined. D-600 at concentrations of 10 to 100 µM did not inhibit or enhance the histamine release induced by 10 µg/ml of PEI6, PEI18 and PAA100, respectively, in 10 min at 37°C (Table 3). W-7 at 5–30 µM had no significant effect on the histamine release induced by PEI6, PEI18 or PAA100 (Table 3).

Thus, the histamine releases induced by PEI6, PEI18 and PAA100 were not inhibited by blockade of calcium channels or inhibition of calmodulin, suggesting that the release is not appreciably dependent on extracellular calcium. To confirm this, we examined the effect of polycations on the intensity of fluorescence of cells loaded with the calcium indicator dye fura-2. After addition of PEI6, PEI18 or compound 48/80, the F340/F380 ratio increased in the presence of extracellular calcium, but did not change in its absence (Fig. 6). PAA100 increased the ratio slowly in the presence or absence of calcium, probably due to its fusogenic activity, not to increase the intracellular concentration of calcium ion.

Effects of cyclic nucleotide on histamine release induced by polycations: Preincubation of mast cells with 1–10 mM dibutyryl cyclic AMP significantly reduced histamine release induced by PEI6, PEI18, PAA100 and compound 48/80 (Fig. 7). On the other hand, preincubation with the same concentrations of 8-bromo cyclic GMP did not inhibit the release induced by PEI6 or PEI18, but inhibited the release induced by compound 48/80 or PAA100 (Fig. 8): 10 mM 8-bromo cyclic GMP inhibited histamine release by PAA100 about 50%. In the absence of polycations, 8-bromo cyclic GMP did not enhance spontaneous histamine release.

Discussion
We found that fusogenic polycations induced histamine release from rat mast cells in the presence of calcium. Acetylated derivatives and triethylentetramine, which are not fusogenic, did not induce histamine release. The potencies of these polymers in inducing histamine release did not depend on their molecular weights, like those of compound...
Fig. 6. Fluorescence intensity ratio in fura-2-loaded mast cells stimulated by PEI₆, PEI₁₈, PAA₁₀₀ and compound 48/80 in the presence and absence of extracellular calcium. Purified mast cells were loaded by incubation in fura-2 acetoxy methylester and stimulated at the time marked by an arrow. Fluorescence of fura-2 was excited alternately by light at 340 and 380 nm. The fluorescence ratio \( F_{340}/F_{380} \) was plotted. Upper record: ratio in the presence of 1 mM calcium. Lower record: ratio in the absence of calcium. A, PEI₆ (10 μg/ml); B, PEI₁₈ (10 μg/ml); C, PAA₁₀₀ (10 μg/ml); D, compound 48/80 (1 μg/ml).

Fig. 7. Effects of dibutyryl cyclic AMP on histamine release induced by PEI₆, PEI₁₈, PAA₁₀₀ and compound 48/80. Purified mast cells were pretreated with dibutyryl cyclic AMP for 10 min and treated with polycation for 10 min. Values are means±S.E. for 3–4 replicate experiments. When no vertical bar is shown, the S.E. was less than 2%. Open symbols indicate spontaneous histamine release. *P<0.05, **P<0.01, ***P<0.001. A, release induced by 10 μg/ml PEI₆; B, release induced by 10 μg/ml PEI₁₈; C, release induced by 10 μg/ml PAA₁₀₀; D, release induced by 1 μg/ml compound 48/80.

48/80 and adrenocorticotropic hormone, but not like those of substance P, mellitin and polylysine (5, 19–22). In contrast, the fusogenic activities of these polycations depended simply on their molecular weights (23). Compound 48/80 and PEIs induced his-
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Fig. 8. Effects of 8-bromo cyclic GMP on histamine release induced by PEI₆, PEI₁₈, PAA₁₀₀ and compound 48/80. Purified mast cells were pretreated with 8-bromo cyclic GMP for 10 min, and treated with polycation for 10 min. Values are means±S.E. for 3 replicate experiments. When no vertical bar is shown, the S.E. was less than 2%. Open symbols indicate spontaneous histamine release. *P<0.05, ***P<0.001. A, release induced by 10 μg/ml PEI₆; B, release induced by 10 μg/ml PEI₁₈; C, release induced by 10 μg/ml PAA₁₀₀; D, release induced by 1 μg/ml compound 48/80.

tamine release in the absence of calcium (Ref. 24, Fig. 2). Drugs that modify calcium movement, such as calcium antagonists and calmodulin inhibitors, inhibit histamine release from mast cells stimulated by concanavalin A, but not by compound 48/80 (14, 25). The histamine release by PEIs was not inhibited by D-600 or W-7. W-7 at 60 μM enhanced histamine release, because it released histamine cytotoxically in the absence of polycations, as reported previously (25). The histamine releases induced by compound 48/80 and PEIs were decreased upon an increase of intracellular cyclic AMP (26) or dibutyryl cyclic AMP (Fig. 7). Thus the histamine releasing mechanisms by PEIs and compound 48/80 seem not to involve calcium channels or calmodulin.

This conclusion is supported by our finding that the ratio of fluorescence of fura-2 was not changed by PEIs or PAAAs in the absence of calcium. As described by Mita and Uchida (27), the calcium indicator dye reduces the availability of intracellular calcium ion, but results showed that polycations release histamine from fura-2 loaded mast cells as well as from the intact mast cells. PEI₆ and PEI₁₈ were able to release histamine in the absence of an increase in the intracellular concentration of free calcium, but PAA₁₀₀ was not. It was interesting that in the presence of extracellular calcium, PEI₆ and PEI₁₈ increased the intracellular concentration of free calcium. This increase was not inhibited by treatment with D-600 (100 μM) for 30 sec (data not shown). This result, which confirmed a previous finding that histamine release induced by PEI₆ or PEI₁₈ was not inhibited by D-600 (Table 3), suggests that the mechanism of calcium influx activated by PEI₆ and PEI₁₈ does not involve innate calcium channels, which are sensitive to D-600. It is possible that there was a slight change in the intracellular concentration of calcium, but this was not detectable using fura-2 because fura-2 penetrates into mast cell granules and is released concomitantly with histamine (28). However, compound 48/80 induced a change in fluorescence of single cells loaded with fura-2 in the absence of extracellular calcium (29), because compound 48/80 enhanced phosphatidylinositol turnover of mast cells and increased the concentration of inositol-1,3,5-trisphosphate, which releases intracellular calcium (30), as well as mastoparan (31). PEIs and PAAAs may also influence intracellular inositol trisphosphate. Our attempts to determine the intracellular concen-
tration of inositol trisphosphate were unsuccessful. 8-Bromo cyclic GMP enhances histamine release from slices of human lung (32). We found that cyclic SMP did not enhance spontaneous histamine release from mast cells, but inhibited histamine release induced by PAA$_{100}$ or compound 48/80. Because it decreases the intracellular concentration of calcium ion in smooth muscle and platelets (33–35), it may control the intracellular concentration of calcium ion in mast cells.

Lactate dehydrogenase was released from mast cells treated with Triton X-100, but not with compound 48/80, because Triton X-100 visibly disrupted surface membranes (6, 36). To determine the release of lactate dehydrogenase, we concluded that PEI$_6$, PEI$_{12}$, PEI$_{18}$ and PEI$_{10}$-OH did not induce perturbation of the membranes of mast cells, but PEI$_{100}$, PEI$_{300}$, PAA$_{30}$-$_{40}$ and PAA$_{100}$ were somewhat cytotoxic. It is interesting, however, that the release of histamine induced by PAAs was greatly reduced in the absence of extracellular calcium, suggesting that this release was partially due to extracellular calcium: in the absence of extracellular calcium, PAAs caused release of more than 60% of the total lactate dehydrogenase, although they released less than 30% of the total histamine. In the absence of calcium, PAAs did not increase the concentration of intracellular calcium, and consequently did not release much histamine. However, addition of calcium shortly after pretreatment with PAA$_{100}$ in the absence of calcium resulted in histamine release. The histamine release induced by PAA$_{100}$ decreased rapidly with time, unlike that induced by compound 48/80: histamine was still released 15 min after stimulation by compound 48/80 (14). The Ca-dependent histamine release induced by PAAs was not inhibited by D-600 or W-7, suggesting that the mechanism of calcium influx by PAAs is different from that stimulated by antigens and/or compound 48/80.

Two types of polycations induce histamine release from mast cells: compounds of one type, such as compound 48/80, are only effective on mast cells, whereas those of the other type that strongly activate phospholipase A$_2$, such as mastoparan and melittin, are effective on both mast cells and platelets (9, 10, 37). We found that PEIs and PAAs were of the former type. Therefore, their histamine-releasing potency was not due to simple perturbation of the mast cell membrane. There seemed to be a membrane receptor for PEIs on mast cells, but not on platelets. The histamine-releasing activity of PEIs was not correlated with their activities in causing fusion of liposomes, suggesting that mast cells have some binding sites for PEIs, which partially overlap those for compound 48/80 (38). Mast cells also have other receptor sites for PAAs, because the mechanisms of histamine release by PEIs and PAAs may be different.

The structures of PEIs and PAAs are simpler than that of compound 48/80 and are easily modified. Moreover, PEIs and PAAs are stable in aqueous solution for at least three months, whereas compound 48/80 in aqueous solution lost its histamine-releasing activity within one day. Thus these polycations should be useful in further studies on the mechanisms of signal transduction by-passing IgE receptors of rat mast cells.

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