Comparison of Histamine Release Induced by Synthetic Polycations with That by Compound 48/80 from Rat Mast Cells

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Abstract—We compared the histamine release induced by polyethylenimines and polyallylamines with that induced by compound 48/80. Lidocaine inhibited the histamine release induced by polyethylenimine with a molecular weight of 600 (PEI_6), but disodium cromoglycate did not. The histamine releases induced by all polyethylenimines and polyallylamines tested were inhibited by lidocaine, but not by disodium cromoglycate. Islet activating protein inhibited the histamine release induced by PEI_6 . Its effects on the release by other polyethylenimines and polyallylamines were less than that on PEI_6 . It is likely that the inhibition of G proteins by islet activating protein resulted in a decrease of the histamine release. This possibility was supported by the finding that guanyl-5'-(β,γ -imino) triphosphate enhanced the histamine release. An inhibitor of polyphosphoinositide phosphodiesterase, neomycin, did not affect the histamine releases induced by these polymers. The effect of PEI_6 seemed to resemble that of compound 48/80. After pretreatment of mast cells with wheat germ agglutinin and with *Limax flavus* agglutinin, releases of histamine induced by PEI_6 and compound 48/80 decreased, suggesting that the binding sites of PEI_6 and compound 48/80 had sialic acid and/or N-acetyl glucosamine residues. The binding site for PEI_6 seemed to especially overlap those of compound 48/80.

Our group previously reported that synthetic polycations with various molecular weights [polyethylenimines (PEIs) and polyallylamines (PAAs), ref. 1] released histamine from rat mast cells (2). PEIs and PAAs are simpler than compound 48/80 in chemical structure and are stable in aqueous solution. Unlike their fusogenic activities (1), histamine-releasing potencies of these polymers were not affected simply by the polymerization, as

was compound 48/80 (3); the histamine releases induced by PEI_6 , PEI_{12} and PEI_{100} were more than those by triethylenetetramine and PEI_{18} , while that by PAA_{30-40} was similar to that of PAA_{100} (2).

There are three types of histamine release inhibitors from rat mast cells: specific inhibitors of histamine release mediated by IgE receptors, such as methoxy verapamil (D-600, a calcium antagonist) and disodium cromoglycate (DSCG) (4, 5); specific inhibitors of release induced by compound 48/80, such as islet activating protein (IAP) (6, 7); and inhibitors of both histamine releases mediated by IgE receptors and compound 48/80, such as dibutyl cyclic AMP and local anesthetics (8–10). The histamine releases induced by PEIs and PAAs were inhibited by dibutyl cyclic AMP, but not by D-600, and D-600

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Abbreviations: PEI_x , polyethylenimine with a molecular weight of ($x \times 100$); PAA_x , polyallylamine with a molecular weight of ($x \times 100$); DSCG, disodium cromoglycate; IAP, islet activating protein; GppNHP, guanyl-5'-(β,γ -imino) triphosphate; Con A, concanavalin A; PHA, phytohemagglutinin; WGA, wheat germ agglutinin; LFA, *Limax flavus* agglutinin.

did not block the increase in intracellular calcium concentrations by polycations (2).

In this study, we compared the effects of lidocaine, DSCG and IAP on histamine releases induced by PEIs and PAAs with those induced by compound 48/80. Mechanisms of action of IAP were further examined using a GTP analogue and neomycin; the GTP analogue released histamine from permeabilized mast cells by ATP or streptolysin-O; and neomycin (an inhibitor of polyphosphoinositide phosphodiesterase) inhibited the release only from the permeabilized cells by ATP (11, 12). We also examined the effects of lectins, which recognize specific sugar residues, on histamine releases induced by polycations and discussed possible receptor sites of polycations and compound 48/80 on mast cells.

Materials and Methods

Preparation of purified mast cells: Mast cells from the peritoneal cavity of male Sprague-Dawley rats, weighing 300–350 g, were purified using Ficoll 400 (Pharmacia) as previously described (4). The purity of the mast cells in the final preparation was more than 90%.

Assay of histamine release from rat mast cells: A 1-ml sample of cell suspension (5×10^4 cells) in Hepes-buffered Tyrode solution was preincubated at 37°C for 10 min and then incubated with polycations for another 10 min. The cells were treated with lidocaine and lectins for 10 min and DSCG for 10 sec before adding the polycation. The mast cells were exposed to IAP by incubating the cell suspension (10^6 cells/ml) in Hepes-buffered Tyrode solution containing various amounts of IAP under 95% O₂/5% CO₂ at 37°C for 2 hr. The cells were then washed twice or three times (150×g, 4°C, 5 min) and resuspended in Hepes-buffered Tyrode solution. Mast cells were permeabilized with 150 μM ATP in the absence of divalent cations (11), and the GTP analogue and neomycin were loaded into the permeabilized mast cells at 37°C for 5 min in Hepes-buffered Tyrode solution, because these molecules were membrane impermeable. To reseal the cells, they were then incubated with 1 mM MgCl₂ at 37°C for 5 min and then washed in Ca-free Hepes-

buffered Tyrode solution. The washed cells were resuspended in Ca-free Hepes-buffered Tyrode solution and preincubated for 5 min. They were then incubated successively with 1 mM CaCl₂ for 5 min.

After treatment with various drugs, the cells were then incubated with polycations (10 μg/ml) or compound 48/80 (1 μg/ml) for 10 min. Ice-cold Hepes-buffered Tyrode solution (1.8 ml) 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 using the fluorometric assay of Shore et al. (13). Histamine release was calculated as a percentage of the total cell content. Values for histamine release are given as means±S.E. for three or four replicate experiments on different samples of pooled cells. As previously described (2), PEIs and PAAs release histamine from intact mast cells dose-dependently. The histamine releases induced by PEI₆, PEI₁₂, PEI₁₈, PEI₁₀₀, PAA₃₀₋₄₀ and PAA₁₀₀ (10 μg/ml) in 10 min at 37°C were 63.1±2.3 (n=19), 66.2±1.2 (n=18), 42.6±2.4 (n=17), 59.1±3.5 (n=21), 64.8±1.7 (n=21) and 62.2±2.4 (n=21) % of the total content, respectively. The spontaneous histamine release was 6.35±0.46% (n=32). Histamine-releasing potencies of polycations (10 μg/ml) were reduced after incubation of the cells in the absence of IAP for 2 hr; the histamine release as a % of the total cell content of these cells stimulated by PEI₆, PEI₁₂, PEI₁₈, PEI₁₀₀, PAA₃₀₋₄₀ and PAA₁₀₀ were 37.8±2.1 (n=6), 40.9±4.7 (n=7), 11.0±2.0 (n=6), 53.1±2.6 (n=7), 53.5±1.7 (n=7) and 51.6±4.0 (n=6) %, respectively. Spontaneous histamine release was not affected by pretreatment of the cells with DSCG, IAP or neomycin. The inhibitory effects of the drugs were calculated by the following equation:

$$\% \text{ Inhibition} = 100 - \frac{[(\text{histamine release with drug}) - (\text{spontaneous release})]}{[(\text{histamine release without drug}) - (\text{spontaneous release})]} \times 100$$

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

Chemicals: Polycations (Fig. 1) were gifts from Drs. N. Oku and M. Nango. The islet ac-

Fig. 1. Structures of polyethylenimine (A) and polyallylamine (B).

tivating protein was from Funakoshi Pharmaceutical Co. (Tokyo, Japan). Guanylyl-5'-(β,γ -imino) triphosphate (GppNHp, tetralithium salt) and ATP were purchased from Boehringer-Mannheim Yamanouchi (Tokyo, Japan) and Wako Pure Chemicals (Osaka, Japan), respectively. Lidocaine HCl and DSCG (Intal®) were gifts from Fujisawa Pharmaceutical Co. (Osaka, Japan). Compound 48/80, neomycin sulfate and concanavalin A (Con A) were purchased from Sigma Chemical Co. (St. Louis, MO). Phytohemagglutinin (PHA) and *Limax flavus* agglutinin (LFA) were purchased from EY Laboratories (San Mateo, CA) and wheat germ agglutinin (WGA) was from Honen Corp. (Tokyo, Japan). Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] was purchased from Dojindo Laboratories (Kumamoto, Japan). Hepes-buffered Tyrode solution had the following composition: 137 mM NaCl, 2.7 mM KCl, 12 mM Hepes, 1 mM MgCl_2 , 1 mM CaCl_2 , 5.6 mM dextrose and 0.03% bovine serum albumin, pH 7.4. Ca-free Hepes-buffered Tyrode solution had the same composition, but without CaCl_2 .

Results

Effects of lidocaine and DSCG on histamine release induced by polycations: The inhibitory effects of lidocaine on histamine releases induced by PEIs and PAAs are shown in Fig. 2 as % inhibition; its IC_{50} values for PEI_6 , PEI_{12} , PEI_{18} , PEI_{100} , PAA_{30-40} and PAA_{100}

Fig. 2. Inhibitory effects of lidocaine on histamine release induced by PEIs and PAAs. Purified mast cells were preincubated with lidocaine for 10 min, and polycation (10 $\mu\text{g}/\text{ml}$) was added. After 10 min, the reaction was stopped and the cell suspension was centrifuged. The histamine content of the supernatant was determined, and the % inhibition was calculated. Values are means $\pm$ S.E. for 6 to 8 replicated experiments. When no vertical bar is shown, the S.E. was less than 2%. ○, PEI_6 ; △, PEI_{12} ; □, PEI_{18} ; ◇, PEI_{100} ; ●, PAA_{30-40} ; ▲, PAA_{100} .

were 12.5, 8.0, 5.2, 19.4, 8.4 and 7.3 mM, respectively. Its inhibitory effects on histamine releases induced by PEI_6 , PEI_{12} and PEI_{100} increased with an increase in its concentration, although the values for histamine release by PEI_{18} , PAA_{30-40} and PAA_{100} reached a plateau at a concentration of 10 mM. Lidocaine (10 mM) also inhibited the histamine release when added only 10 sec before the cells were exposed to polycations (data not shown). Spontaneous histamine release in the presence of 10 mM or 30 mM lidocaine were $11.6 \pm 1.9\%$ ($n=8$) and $14.4 \pm 2.1\%$ ($n=14$), respectively. The inhibitory effects of 10 mM lidocaine on the histamine releases induced by PEI_6 , PEI_{12} , PEI_{100} , PAA_{30-40} and PAA_{100} were partly reversed by the addition of a polycation (Fig. 3).

We confirmed that histamine release mediated by IgE receptors was inhibited by DSCG; the IC_{50} value for DSCG was 3.2 μM . However, DSCG (3 μM) did not inhibit the histamine releases induced by PEI_6 , PEI_{12} , PEI_{18} , PEI_{100} , PAA_{30-40} and PAA_{100} ; the % inhibitions were 9.5 ± 5.5 , 9.8 ± 5.5 , $27.9 \pm$

Fig. 3. Effects of lidocaine on histamine release induced by various amounts of PEIs and PAAs. Purified mast cells were preincubated with 10 mM lidocaine for 10 min, and then the polycation (1–10 $\mu\text{g/ml}$) was added. After 10 min, the reaction was stopped and the cell suspension was centrifuged. The histamine content of the supernatant was determined. Values are means $\pm$ S.E. for 6 to 8 replicated experiments. When no vertical bar is shown, the S.E. was less than 2%. A, PEI_6 ; B, PEI_{12} ; C, PEI_{18} ; D, PEI_{100} ; E, PAA_{30-40} ; F, PAA_{100} . Closed and open circles indicate values in the presence and absence of 10 mM lidocaine, respectively.

11.2, 6.2 ± 10.3 , 12.2 ± 12.6 and $24.0 \pm 11.7\%$, respectively ($n=6$). Lidocaine inhibited the histamine release induced by compound 48/80 with an IC_{50} of 15 mM, but DSCG did not (data not shown).

Effects of IAP on histamine releases induced by polycations: After preincubation of mast cells with IAP, the histamine releases induced by PEI_6 , PEI_{12} , PEI_{100} , PAA_{30-40} and PAA_{100} were significantly decreased (Fig. 4). IAP inhibited the histamine release induced by PEI_6 and PEI_{12} , as well as that by compound 48/80, although it had less of an

Fig. 4. Effects of IAP on histamine release induced by PEIs and PAAs. Purified mast cells were preincubated with IAP under 95% O_2 /5% CO_2 at 37°C for 2 hr. The cells were washed twice or three times and resuspended in fresh Hepes-buffered Tyrode solution (pH 7.4) and exposed to polycation (10 $\mu\text{g/ml}$). After 10 min, the reaction was stopped and the cell suspension was centrifuged. The histamine content of the supernatant was determined. Values are means $\pm$ S.E. When no vertical bar is shown, the S.E. was less than 2%. A, PEI_6 ($n=6$); B, PEI_{12} ($n=7$); C, PEI_{18} ($n=5$); D, PEI_{100} ($n=7$); E, PAA_{30-40} ($n=7$); F, PAA_{100} ($n=6$). Closed circles indicate histamine release induced by PEIs and PAAs, and open circles indicate spontaneous histamine release. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

effect on the histamine releases induced by PEI_{100} , PAA_{30-40} and PAA_{100} . We confirmed the effect of IAP on the release by compound 48/80; the histamine releases induced by 1 $\mu\text{g/ml}$ compound 48/80 in 10 min from mast cells preincubated with 0, 0.1, 0.3, 1 and 3 ng/ml of IAP were 48.2 ± 3.8 , 45.9 ± 4.8 , 36.6 ± 2.5 ($P<0.05$), 20.4 ± 2.3 ($P<0.01$) and 18.4 ± 1.8 ($P<0.01$) % of the total cell content, respectively ($n=6$). Histamine release induced by PEI_{18} was not affected by IAP. It is noteworthy that histamine-releasing activities of polycations, especially PEI_{18} , were reduced after incubation of the cells in the absence of IAP for 2 hr.

Effects of GppNHp and neomycin on histamine releases induced by polycations: When the cells were permeabilized with ATP in the

Fig. 5. Effects of GppNHP on histamine release induced by PEIs and PAAs. GppNHP (1 mM) was loaded into permeabilized mast cells in the presence of 150 μ M ATP or in the absence of divalent cations at 37°C for 5 min. The cells were resealed by adding 1 mM $MgCl_2$ at 37°C for 5 min and washed in Ca-free Hepes-buffered Tyrode solution. The washed cells were resuspended and preincubated for 5 min. They were incubated with 1 mM $CaCl_2$ for 5 min and then with polycation for 10 min at 37°C. Values are means $\pm$ S.E. for 6 replicated experiments. When no vertical bar is shown, the S.E. was less than 2%. A, PEI_6 ; B, PEI_{12} ; C, PEI_{18} ; D, PEI_{100} ; E, PAA_{30-40} ; F, PAA_{100} . Closed circles indicate histamine release from mast cells pretreated with 1 mM GppNHP, and open circles indicate control histamine release in the absence of GppNHP.

absence of divalent cations and resealed with $MgCl_2$, the amounts of histamine released by the polycations decreased (Fig. 5, open circles). With permeabilized mast cells, histamine releases induced by 10 μ g/ml PEI_6 , PEI_{100} , PAA_{30-40} and PAA_{100} were less than those by 1 μ g/ml of the respective polycations (Fig. 5, open circles), while with intact mast cells, 1 μ g/ml polycations induced no appreciable histamine release (Fig. 3, open circles). GppNHP at 1 mM significantly in-

creased histamine release in the absence of the polycations and enhanced histamine release in the presence of polycations (Fig. 5). The enhancement of histamine release by GppNHP in the presence of PEI_6 and PEI_{100} increased with an increase in the concentration of the polycations, while those by PAAs did not. PAAs produced a pattern of bell-shaped release of histamine in the presence and absence of GppNHP.

The releases of histamine by PEIs and PAAs from resealed mast cells pretreated with neomycin are shown in Table 1. Neomycin did not inhibit or enhance the histamine releases induced by PEIs and PAAs; the differences in the release with and without neomycin cannot be regarded as significant.

Effects of lectins on histamine release induced by PEI_6 , PAA_{100} and compound 48/80: PEI_6 was studied further because it resembled compound 48/80 as shown in Fig. 4. Effects of Con A, PHA and WGA on the histamine releases induced by PEI_6 , PAA_{100} and compound 48/80 are shown in Table 2. The release induced by 3 μ g/ml PEI_6 was inhibited by WGA and PHA, but not by Con A. LFA, a specific lectin for sialic acid, also decreased the release of histamine induced by PEI_6 (3 μ g/ml) from $41.1 \pm 2.0\%$ ($n=6$) (in the absence of LFA) to $13.5 \pm 3.0\%$ ($n=6$) (in the presence of 100 μ g/ml LFA) of the total cell content. Moreover, the release of histamine induced by compound 48/80 was inhibited by WGA and PHA, but not by Con A; % inhibition of 100 μ g/ml WGA on histamine release induced by 0.3 μ g/ml and 1 μ g/ml compound 48/80 were $52.0 \pm 6.0\%$ ($n=12$) and $30.4 \pm 2.2\%$ ($n=6$), respectively. It is noteworthy that Con A, PHA and WGA inhibited the histamine release induced by PAA_{100} . Under our conditions, Con A, PHA and WGA did not release histamine solely: The histamine release percents in the presence of 100 μ g/ml Con A, PHA and WGA were $4.25 \pm 0.28\%$, $5.07 \pm 0.50\%$ and $3.62 \pm 0.50\%$ ($n=6$), respectively.

Discussion

We found that lidocaine inhibited histamine release induced by PEIs and PAAs. An inhibitory effect of lidocaine on the release was observed only after 10 sec incubation with mast

Table 1. Effects of neomycin on histamine release induced by polycations

Polycation	($\mu\text{g/ml}$)	Histamine release % from mast cells pretreated		P
		without neomycin	with neomycin	
PEI ₆	0	8.0 $\pm$ 0.8	8.3 $\pm$ 1.3	>0.1
	1	15.7 $\pm$ 1.0	20.0 $\pm$ 2.3	>0.1
PEI ₁₂	1	16.6 $\pm$ 1.4	15.9 $\pm$ 1.7	>0.1
PEI ₁₈	1	15.5 $\pm$ 2.7	23.0 $\pm$ 3.7	>0.1
PEI ₁₀₀	1	18.4 $\pm$ 1.2	22.0 $\pm$ 1.0	>0.05
PAA ₃₀₋₄₀	1	23.3 $\pm$ 4.3	25.3 $\pm$ 1.7	>0.1
PAA ₁₀₀	1	25.4 $\pm$ 3.0	19.3 $\pm$ 2.0	>0.1

Neomycin (100 μM) was loaded into permeabilized mast cells at 37°C for 5 min in Hepes-buffered Tyrode solution (pH 7.4) with 150 μM ATP in the absence of divalent cations. The cells were then resealed by adding 1 mM MgCl₂ at 37°C for 5 min and washed in Ca-free Hepes-buffered Tyrode solution (pH 7.4). They were then incubated successively with 1 mM CaCl₂ for 5 min and with polycation for 10 min at 37°C. Values are given as means $\pm$ S.E. (n=4).

Table 2. Percent inhibition of histamine release induced by PEI₆, PAA₁₀₀ and compound 48/80 from mast cells pretreated with lectins

Releaser	$\mu\text{g/ml}$	Con A $\mu\text{g/ml}$			(n)
		1	10	100	
PEI ₆	3	7.4 $\pm$ 1.6	2.0 $\pm$ 4.8	9.6 $\pm$ 3.6	(6)
PAA ₁₀₀	3	10.3 $\pm$ 2.7**	27.0 $\pm$ 3.0***	37.6 $\pm$ 2.3***	(6)
Compound 48/80	1	-4.1 $\pm$ 2.3	-0.3 $\pm$ 5.1	9.1 $\pm$ 4.4	(6)
	0.3	-10.0 $\pm$ 4.7	-11.1 $\pm$ 3.4	6.2 $\pm$ 3.3	(12)
	$\mu\text{g/ml}$	PHA $\mu\text{g/ml}$			(n)
		1	10	100	
PEI ₆	3	8.2 $\pm$ 4.0	18.5 $\pm$ 5.8	50.9 $\pm$ 4.5***	(6)
PAA ₁₀₀	3	14.9 $\pm$ 2.8***	17.7 $\pm$ 2.0***	29.7 $\pm$ 1.9***	(6)
Compound 48/80	1	-2.8 $\pm$ 2.9	8.8 $\pm$ 2.4*	1.1 $\pm$ 5.0	(6)
	0.3	14.2 $\pm$ 5.3*	18.4 $\pm$ 2.2***	25.0 $\pm$ 4.1***	(6)
	$\mu\text{g/ml}$	WGA $\mu\text{g/ml}$			(n)
		1	10	100	
PEI ₆	3	8.2 $\pm$ 3.8	27.1 $\pm$ 2.3***	72.7 $\pm$ 3.0***	(6)
PAA ₁₀₀	3	14.9 $\pm$ 7.0	26.1 $\pm$ 2.8***	47.5 $\pm$ 3.0***	(6)
Compound 48/80	1	-3.7 $\pm$ 2.1	2.0 $\pm$ 4.6	30.4 $\pm$ 2.2***	(6)
	0.3	3.7 $\pm$ 3.2	28.8 $\pm$ 6.1***	52.0 $\pm$ 6.0***	(12)

After preincubation with various lectins for 10 min in Hepes-buffered Tyrode solution (pH 7.4), the mast cells were incubated with PEI₆, PAA₁₀₀ and compound 48/80 for 10 min at 37°C. Percent inhibition was calculated as described in Materials and Methods. Values are given as means $\pm$ S.E. *P<0.05, **P<0.01, ***P<0.001.

cells, like the case of the release induced by compound 48/80 (10). Its effects on mast cells did not change the histamine release induced by polycations with various degrees of polymerization (Fig. 2). Its inhibitory effects were partly reversed by a polycation (Fig. 3). Thus, the effects of lidocaine on the histamine release induced by polycations are similar to

that on the release induced by compound 48/80. One of the mechanisms for the inhibitory effect of lidocaine on histamine release induced by Con A was due to inhibition of calcium influx into mast cells (14). However, lidocaine inhibits the histamine release induced by calcium ionophore A23187 when there is enough intracellular calcium to trigger

histamine releases, suggesting that their inhibitory actions on histamine release induced by A23187 cannot be explained by their effect on calcium influx (14). Our group previously reported that the inhibitory effects of lidocaine on mast cells was correlated with the concentration of nonionized molecules which penetrate the mast cell membrane (10). The site of action for lidocaine on mast cells is still unknown, but lidocaine may interfere with steps in the release cascade that occurred after opening of calcium channels, such as calmodulin-dependent ones (15).

DSCG inhibits calcium influx into the mast cells to inhibit histamine release induced by antigens (5), because it binds to cromolyn-binding protein, which is thought to be a component of the innate calcium channels of mast cells (16, 17). DSCG, however, did not inhibit histamine release induced by PEIs or PAAs. Thus an inhibitor of both histamine releases induced by antigens and compound 48/80 inhibited the release induced by PEIs and PAAs, although a specific inhibitor of histamine release induced by antigens did not.

Histamine is released from permeabilized mast cells on activation of their G protein by GTP- γ -S [guanosine-5'-O-(3-thiotriphosphate)] or GppNHp (11, 12). This activation is inhibited by pretreatment of the mast cells with IAP, which also inhibits histamine release from basophils (6, 18, 19), although cholera toxin potentiates serotonin release from basophil leukemia cells, but does not affect histamine release from mast cells (20, 21). We found that IAP inhibited histamine release induced by all the polycations we tested, except PEI₁₈, suggesting that the histamine releases induced by some polycations seemed to be mediated by G protein, like the release by compound 48/80 (Fig. 4). This was confirmed in the experiments using GppNHp (Fig. 5). In the absence of GppNHp, PEI₆, PEI₁₀₀, PAA₃₀₋₄₀ and PAA₁₀₀ produced the pattern of bell-shaped histamine release, like anti-IgE (22). Only PAAs produced the pattern of bell-shaped histamine release in the presence and absence of GppNHp, suggesting that interactions of PAAs with mast cells were somewhat different from those of PEIs.

Gomperts and Fewtrell (23) reported that GDP- β -S [guanosine-5'-O-(2-thiodiphos-

phate)] is an inhibitor of secretion when introduced through a patch pipette, but that it behaves as an activator when trapped in the cytosol by permeabilization and resealing. Our preliminary data showed that GDP- β -S also activated the histamine releases by PEIs and PAAs from permeabilized mast cells, and that when loaded into mast cells at 1 mM concomitantly with GppNHp, it did not inhibit enhancement by GppNHp of histamine release by PEIs and PAAs. It is interesting that GppNHp increases histamine release induced by PEI₁₈, although IAP did not inhibit histamine release induced by PEI₁₈. It remains possible that PEI₁₈ activates G proteins and triggers the release of histamine.

Neomycin at 100 μ M inhibits histamine release and polyphosphoinositide phosphodiesterase of mast cells permeabilized by ATP (12), but did not inhibit them in the cells permeabilized by streptolysin-O (24). Since it did not inhibit the histamine release induced by PEIs and PAAs (Table 1), we supposed that PEIs and PAAs can trigger histamine release by by-passing the activation of polyphosphoinositide phosphodiesterase. However, our attempts to determine the intracellular concentration of inositol triphosphate were unsuccessful.

Secretion from platelets is also dependent on G protein (25), but PEIs and PAAs did not activate serotonin release from rat platelets (2). We then supposed that mast cells have some binding sites for PEIs and PAAs. A mast cell has the binding sites for compound 48/80 on its cell membrane (26). The binding site may be composed of proteins (27) with sialic acid (28). The binding sites for PEIs and/or PAAs may partially overlap those for compound 48/80. To confirm, we examined the effects of lectins on histamine release induced by PEI₆, PAA₁₀₀ and compound 48/80. Lectins recognize specific sugar residues: Con A is specific for mannoside; PHA, for N-acetyl galactosamine; and WGA, for N-acetyl glucosamine and sialic acid. Con A and WGA induced release of histamine potentiated by phosphatidylserine (29, 30). Under our conditions, however, no appreciable release of histamine was observed in the presence of the lectins tested. The concentration of lectins we used seemed to be

enough to interfere with the interaction of polycations with mast cells; at the concentration of 100 $\mu\text{g/ml}$ of Con A, PHA and WGA, there are 2.32×10^8 , 2.02×10^8 and 5.56×10^8 molecules of lectin/mast cell, respectively.

Experimental data led to the hypothesis that the binding sites of PEI_6 and compound 48/80 have sugar residues of sialic acid, N-acetyl glucosamine and/or N-acetyl galactosamine; and those of PAA_{100} have sialic acid, N-acetyl glucosamine, N-acetyl galactosamine and mannose. The binding of PEI_6 to mast cells seemed to be inhibited by WGA due to its affinity to sialic acid rather than to its affinity to N-acetyl glucosamine, because LFA that is specific for sialic acid reduced histamine release induced by PEI_6 . PAA_{100} can bind to the binding sites of compound 48/80, but it can also bind to the other binding sites with mannose. Table 2 shows that PEI_6 interacted with sialic acid and/or N-acetyl galactosamine of the binding sites of compound 48/80, but not with mannose of Con A-receptor, including IgE receptors (31). Therefore, the mechanisms of histamine release induced by PEI_6 resembled those by compound 48/80 rather than those by antigens. The binding of compound 48/80 or PEI_6 to their binding sites of mast cells seemed to activate G protein to release histamine from the mast cells. However, it is not clear whether the binding sites for compound 48/80 and PEI_6 are coupled to G protein of the mast cells directly or secondarily.

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