Inhibitory Effects of Sialic Acid- or *N*-Acetylglucosamine-Specific Lectins on Histamine Release Induced by Compound 48/80, Bradykinin and a Polyethylenimine in Rat Peritoneal Mast Cells

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ABSTRACT—The effects of seven lectins with various sugar-specificities on histamine release from rat peritoneal mast cells induced by non-immunologic stimuli were studied. The non-immunologic stimuli used were three basic secretagogues, compound 48/80, bradykinin and PEI₆ (polyethylenimine with a molecular weight of 600). In this study, we observed inhibition of the histamine release by *Macckia amurensis* mitogen and *Solanum tuberosum* agglutinin (100 µg/ml at 37°C for 10 min), which are specific for sialic acid- α 2,3-*N*-acetyl galactosamine (Sia α 2,3GalNAc) and *N*-acetyl glucosamine (GlcNAc) oligomers, respectively. The effects of *Phytolacca americana* mitogen and *Sambucus sieboldiana* agglutinin were different. Three lectins specific for mucin type oligosaccharides inhibited the histamine release induced by compound 48/80 but not that induced by bradykinin or PEI₆. Since bradykinin and PEI₆ additively enhanced the histamine release induced by compound 48/80, they partially shared the same signalling pathways. Glycoproteins with bisecting GlcNAc and Sia residues, as described previously (Jpn. J. Pharmacol. 57, 79–90, 1991), seemed to be one of the action sites for compound 48/80, bradykinin and PEI₆. In addition to the direct activation of the pertussis toxin-sensitive G proteins, we propose another mechanism of non-immunologic stimuli via specific glycoproteins on rat peritoneal mast cells. The apparent sugar residues involved were asparagine-linked oligosaccharides with Sia (especially Sia α 2,3Gal), GlcNAc oligomers and/or bisecting GlcNAc.

Keywords: Mast cell, Histamine release, Lectin, Non-immunologic stimuli, Sugar-specificity

Rat peritoneal mast cells are a model system for the study of human skin mast cells as both rat peritoneal mast cells (connective tissue type) and human skin mast cells (MC_{TC}) are activated in the presence of non-immunologic stimuli such as anaphylatoxin, compound 48/80, substance P, bradykinin and PEI₆ (1–9). Unlike IgE-dependent activation, the activation by non-immunologic stimuli is independent of IgE receptors, extracellular calcium and phosphatidylserine (10), but is dependent on Gi-like G protein activation (11–14). Compound 48/80, substance

P and bradykinin are basic secretagogues. Since compound 48/80, substance P and bradykinin stimulate the GTPase activity of Gi α because of the interaction between these basic compounds and the negatively charged region of Gi α (15), they seemed to activate mast cells by direct G-protein activation, rather than by a receptor-dependent pathway (16).

Recently our group reported that a 10-min incubation of rat peritoneal mast cells with WGA or PHA-E₄, but not with Con A or PHA-L₄, resulted in sugar-specific inhibition of the histamine release induced by compound 48/80, bradykinin and PEI₆ (14, 17). Glycoproteins were

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Abbreviations used are: PEI₆, polyethylenimine with a molecular weight of 600; Gi, pertussis toxin-sensitive heterotrimeric GTP-binding protein (an inhibitor of adenylate cyclase); WGA, wheat germ agglutinin; PHA-E₄, phytohemagglutinin-E subunits; Con A, Concanavalin A; PHA-L₄, phytohemagglutinin-L subunits; GlcNAc, *N*-acetyl glucosamine; Sia, sialic acid; GalNAc, *N*-acetyl galactosamine; MAM, *Macckia amurensis* mitogen; PWM, *Phytolacca americana* mitogen; SJA, *Sophora japonica* agglutinin; SSA, *Sambucus sieboldiana* agglutinin; STA, *Solanum tuberosum* agglutinin; ABA, *Agaricus bisporus* agglutinin; PNA, *Arachis hypogaea* agglutinin; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; IC₅₀, the concentration producing 50% inhibition; IC₃₀, the concentration producing 30% inhibition.

detected by WGA- and PHA-E₄-blotting, but glycolipids were not (17). Because WGA and PHA-E₄ are GlcNAc-specific, especially the bisecting GlcNAc-specific lectins, glycoproteins with GlcNAc-residues seemed to be involved in the histamine releasing mechanisms, and the binding of WGA and PHA-E₄ to the glycoproteins resulted in inhibition of histamine release. It was not likely that WGA and PHA-E₄ inhibited the interaction between basic secretagogues and α subunit of Gi (Gi α), because Gi α was not detected by WGA- and PHA-E₄-blotting (17). In addition to the direct G protein activation (16), we propose another mechanism of non-immunologic mast cell activation via specific glycoproteins containing GlcNAc oligomers.

WGA and PHA-E₄ also exhibit the ability to bind to a cluster of Sia and GalNAc, respectively. Therefore, in this study, we further examined the inhibitory effects of other lectins on histamine release induced by compound 48/80, bradykinin and PEI₆. The seven lectins tested have different sugar specificities. MAM, PWM, SJA, SSA and STA bind asparagine-linked oligosaccharides; STA and PWM are specific for GlcNAc oligomers, and MAM and SSA are specific for Sia α 2,3Gal and Sia α 2,6Gal, respectively (18–21). PWM also has an affinity to *N*-acetylactosamine (22). ABA, PNA and SJA bind mucin type oligosaccharides with the Gal β 1,3GalNAc residue (22–24). SJA is characterized as a lectin having affinities both to mucin type and asparagine-linked oligosaccharides, and it inhibited histamine release mediated by IgE and compound 48/80, but its inhibition was not sugar-specific because 10 mM L-rhamnose, D-fucose, D-galactose and β -lactose failed to protect the mast cells from inhibition by SJA (24, 25). Under our conditions, ABA, MAM, PNA, PWM, SSA and STA did not release histamine from rat peritoneal mast cells. We, therefore, examined the effects of these lectins on histamine release induced by non-immunologic stimuli from rat peritoneal 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 in HEPES-buffered Tyrode solution (137 mM NaCl, 2.7 mM KCl, 12 mM HEPES, 1 mM MgCl₂, 0.3 mM CaCl₂, 5.6 mM dextrose and 0.03% bovine serum albumin, pH 7.4) by using Ficoll 400 (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) as previously described (26). The purity of the mast cells in the final preparation was more than 90%.

Assay of histamine release from rat mast cells

A 0.5-ml sample of cell suspension (2×10^4 cells) in

HEPES-buffered Tyrode solution was treated with and without lectins at 37°C for 10 min. After treatment with lectins, the cells were then incubated with compound 48/80 (0.3 μ g/ml), bradykinin (50 μ M) or PEI₆ (3 μ g/ml) at 37°C for 10 min. In the case of A23187-induced histamine release, the mast cells were challenged with A23187 (1 μ M) at 37°C for 15 min. Ice-cold HEPES-buffered Tyrode solution (2.3 ml) was added to terminate the reaction, and the mixture was centrifuged at $2,100 \times g$ for 10 min at 4°C. Released histamine in the supernatant was determined by the fluorometric assay of Shore et al. (27). Since A23187 interferes with the fluorometric assay, it was removed from the samples before the determination, as previously described (28). Histamine release was calculated as a percentage of the total cell content. Total histamine content was measured after treatment of the cells in the presence of 0.03 N HCl at 100°C for 3 min. Values for histamine release are given as the means $\pm$ S.E. for several replicate experiments on different samples of pooled cells. The histamine release percentages induced by compound 48/80, bradykinin and PEI₆ at 37°C for 10 min were 58.4 ± 1.7 (n=52), 43.5 ± 1.5 (n=38), and 41.0 ± 1.5 (n=41) % of the total content, respectively. Spontaneous histamine release at 37°C for 10 min in the absence of basic secretagogues was 5.9 ± 0.2 (n=100) %. No appreciable release of histamine was observed in the presence of tested lectins. The net histamine release % and the inhibitory effects of the lectins were calculated by the following equations, respectively:

$$\text{Net histamine release \%} = (\text{histamine release \%})$$

$$- (\text{spontaneous histamine release \%})$$

$$\% \text{ Inhibition} = 100 - (\text{histamine release with lectin}$$

$$- \text{spontaneous release}) / (\text{histamine release without lectin}$$

$$- \text{spontaneous release}) \times 100$$

Treatment of neuraminidase

Purified mast cells were treated with neuraminidase (5×10^{-4} U/ml) at 37°C for 1 hr with gentle shaking. The cells were washed twice, resuspended in fresh HEPES-buffered Tyrode solution and exposed to A23187 (1 μ M) or compound 48/80 (0.3 μ g/ml). There was no detectable protease activity in the neuraminidase preparation.

SDS-PAGE and lectin-blotting

SDS-PAGE and lectin-blotting were carried out as described previously (17). The cell pellets (4×10^7 cells/ml) were lysed in the presence of protease inhibitors. Protein extracts were fractionated by SDS-PAGE on 7.5% acrylamide gel and transferred to the nitrocellulose paper. Glycoproteins were detected with biotinyl-MAM and avidine-conjugated horseradish peroxidase after blocking with BSA. To examine the effect of neuraminidase, mast cells were incubated with neuraminidase (10^{-2}

U/ml) at 37°C for 1 hr, washed twice and then lysed in the presence of protease inhibitors.

Statistical analyses

Statistical significance of the histamine release %, net histamine release % or % inhibition was evaluated by the unpaired Student's *t*-test, with $P < 0.05$ being regarded as significant.

Chemicals

Compound 48/80 and bradykinin (acetate salt) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). A23187 and neuraminidase (*Clostridium perfringens*) were from Calbiochem, Japan (Tokyo) and Boehringer-Mannheim Yamanouchi (Tokyo), respectively. PEI₆ was a gift from Drs. N. Oku and M. Nango. ABA, MAM, PNA, PWM, SSA and biotinyl-MAM were purchased from the Honen Corp. (Tokyo). SJA and STA were from EY Laboratories (San Mateo, CA, USA). The avidin-conjugate of horseradish peroxidase was from Zymed (San Francisco, CA, USA).

RESULTS

Effects of MAM and STA on histamine release induced by compound 48/80, bradykinin and PEI₆

The histamine release induced by compound 48/80, bradykinin and PEI₆ were inhibited by incubation of the cells with the Sia-specific lectin MAM for 10 min (Fig. 1).

IC₅₀ values of MAM of histamine release induced by compound 48/80, bradykinin and PEI₆ were 27.2, 43.1 and 42.9 µg/ml, respectively. Its inhibitory effect did not increase at concentrations of 200 and 300 µg/ml (data not shown).

GlcNAc oligomer-specific lectin STA inhibited the histamine release induced by bradykinin and PEI₆ (Fig. 1), IC₃₀ values being 76 and 17.5 µg/ml, respectively. The inhibitory effect of STA on histamine release induced by compound 48/80 was significant, but not strong enough to enable calculation of its IC₃₀ value. STA effectively inhibited the histamine release induced by PEI₆ with an IC₅₀ value of 73.9 µg/ml. GlcNAc (30 mM) partially reversed the inhibition of histamine release induced by PEI₆; percentages of inhibition by STA (100 µg/ml) in the absence and presence of GlcNAc (30 mM) were 54.0 ± 2.0 ($n=8$) and 34.5 ± 2.7 ($n=6$, $P < 0.01$) %, respectively.

On the other hand, MAM and STA did not inhibit the histamine release induced by A23187. Percentages of histamine release inhibition induced by A23187 (1 µM) in the presence of MAM (100 µg/ml) and STA (100 µg/ml) were -2.4 ± 6.2 ($n=6$) and -18.3 ± 9.8 ($n=6$) %, respectively. The A23187 (1 µM) induced histamine release from neuraminidase (5×10^{-4} U/ml)-treated mast cells (% inhibition $< 2\%$) was not significantly different from that of the untreated cells. On the other hand, the neuraminidase (5×10^{-4} U/ml) treatment of mast cells inhibited the histamine release induced by compound 48/80 (0.3 µg/ml) by 43.1 ± 3.6 ($n=6$, $P < 0.001$) %.

Fig. 1. Effects of STA and MAM on histamine release induced by compound 48/80, bradykinin or PEI₆. After preincubation with STA (open circles) or MAM (closed circles) for 10 min, the mast cells were incubated with compound 48/80 (0.3 µg/ml), bradykinin (50 µM) or PEI₆ (3 µg/ml) for 10 min at 37°C. Percent inhibition was calculated as described in Materials and Methods. Values are given as the means $\pm$ S.E. for 6 to 8 replicate experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 2. Effects of SSA (A) and PWM (B) on histamine release induced by compound 48/80, bradykinin or PEI_6 . After preincubation with SSA or PWM for 10 min, the mast cells were incubated with compound 48/80 (0.3 $\mu\text{g/ml}$, open circles), bradykinin (50 μM , closed triangles) or PEI_6 (3 $\mu\text{g/ml}$, closed circles) for 10 min at 37°C. Percent inhibition was calculated as described in Materials and Methods. Values are given as the means $\pm$ S.E. for 6 to 8 replicate experiments. * $P < 0.05$, *** $P < 0.001$.

Effects of SSA and PWM on the histamine release induced by compound 48/80, bradykinin and PEI_6

Since MAM recognizes $\text{Sia}\alpha 2,3\text{Gal}$, we further examined the effects of SSA which is more specific for $\text{Sia}\alpha 2,6\text{Gal}$ than $\text{Sia}\alpha 2,3\text{Gal}$. SSA inhibited the histamine release induced by compound 48/80 ($\text{IC}_{30} = 19.6 \mu\text{g/ml}$, $\text{IC}_{50} = 53.0 \mu\text{g/ml}$) and bradykinin ($\text{IC}_{30} = 75.1 \mu\text{g/ml}$),

but not that induced by PEI_6 (Fig. 2A). On the other hand, the histamine release induced by PEI_6 was significantly inhibited by the GlcNAc oligomer- and *N*-acetyl lactosamine-specific lectin PWM with an IC_{50} of 47.2 $\mu\text{g/ml}$, whereas those induced by compound 48/80 and bradykinin were not inhibited (Fig. 2B). Preincubation of PWM with its inhibitory sugar, chitooligosaccharide, at

Fig. 3. Effects of PNA and ABA on histamine release induced by compound 48/80, bradykinin or PEI_6 . After preincubation with PNA (open circles) or ABA (closed circles) for 10 min, the mast cells were incubated with compound 48/80 (0.3 $\mu\text{g/ml}$), bradykinin (50 μM) or PEI_6 (3 $\mu\text{g/ml}$) for 10 min at 37°C. Percent inhibition was calculated as described in Materials and Methods. Values are given as the means $\pm$ S.E. for 5 to 14 replicate experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 4. Effects of SJA on histamine release induced by compound 48/80, bradykinin or PEI₆. After preincubation with SJA for 10 min, the mast cells were incubated with compound 48/80 (0.3 μg/ml, open circles), bradykinin (50 μM, closed triangles) or PEI₆ (3 μg/ml, closed circles) for 10 min at 37°C. Percent inhibition was calculated as described in Materials and Methods. Values are given as the means ± S.E. for 6 to 8 replicate experiments. **P < 0.01, ***P < 0.001.

Fig. 5. Blotting analysis of glycoproteins from rat peritoneal mast cells having affinity to MAM. Glycoproteins were separated on a 7.5% acrylamide gel. Purified mast cells were incubated with neuraminidase (10^{-2} U/ml) at 37°C for 1 hr with gentle shaking. The cells were washed twice and resuspended in fresh HEPES-buffered Tyrode solution. Lane 1, glycoproteins from neuraminidase-untreated mast cells detected using biotinyl-MAM (20 μg/ml); lane 2, glycoproteins from neuraminidase-treated mast cells detected using biotinyl-MAM (20 μg/ml).

the concentration of 1 w/v% had no effect on the inhibition by PWM on the histamine release induced by PEI_6 , its percentage of inhibition being less than 1%.

Effects of lectins that bind mucin type oligosaccharides on the histamine release induced by compound 48/80, bradykinin and PEI_6

ABA and PNA slightly inhibited the histamine release induced by compound 48/80 and had no appreciable effect on that induced by PEI_6 (Fig. 3). PNA (10 $\mu\text{g/ml}$) inhibited the histamine release induced by bradykinin ($P < 0.05$), although not in a dose-dependent manner. The inhibitory effect of ABA on histamine release induced by compound 48/80 was also not dose-dependent (Fig. 3).

Moreover, SJA, which binds to both mucin type and asparagine-linked oligosaccharides, inhibited the histamine release induced by compound 48/80 in a dose-dependent manner, its IC_{50} being 37.8 $\mu\text{g/ml}$. On the other hand, SJA had no appreciable effect on the histamine release induced by bradykinin or PEI_6 (Fig. 4).

Analysis of rat mast cell glycoproteins with affinity to MAM

The glycoproteins of rat peritoneal mast cells were examined with biotinyl-MAM. Figure 5 shows that there are glycoproteins having affinity to MAM, and that the Sia-residues of these glycoproteins were eliminated by treatment of the mast cells with neuraminidase (10^{-2} U/ml, 1 hr).

Additive effects of compound 48/80, bradykinin and PEI_6

We examined the histamine release in the presence of different combinations of low concentrations of compound 48/80 (0.1 $\mu\text{g/ml}$), bradykinin (20 μM) and PEI_6 (1 $\mu\text{g/ml}$). The percentages of net histamine release induced by compound 48/80 (0.1 $\mu\text{g/ml}$), bradykinin (20 μM) and PEI_6 (1 $\mu\text{g/ml}$) were 4.5 ± 1.1 ($n=8$), 4.7 ± 1.5 ($n=5$) and 10.7 ± 1.5 ($n=6$) %, respectively. Spontaneous histamine release in these experiments was 6.1 ± 0.4 ($n=18$) %. Bradykinin (20 μM) and compound 48/80 (0.1 $\mu\text{g/ml}$) together additively enhanced the net histamine release. The same effects were demonstrated with PEI_6 (1 $\mu\text{g/ml}$) and compound 48/80 (0.1 $\mu\text{g/ml}$) and with PEI_6 (1 $\mu\text{g/ml}$) and bradykinin (20 μM) (Table 1).

DISCUSSION

Our group previously reported inhibitory effects of WGA and PHA- E_4 on histamine release induced by non-immunologic stimuli (17). In this study, we found that STA was also inhibitory, like WGA and PHA- E_4 (Fig. 1). Since STA has an affinity to GlcNAc oligomers, it possibly binds to some of the glycoproteins with GlcNAc-

Table 1. Additive effects of three non-immunologic stimuli in different combinations on enhancement of histamine release

Non-immunologic stimuli	Net histamine release (%)
Net histamine release % induced by compound 48/80 and bradykinin	6.3 ± 0.9^A ($n=12$)
Net histamine release % induced by compound 48/80 + net histamine release % induced by bradykinin	8.0 ± 2.1^B ($n=6$)
Net histamine release % induced by compound 48/80 and PEI_6	19.8 ± 2.3^C ($n=8$)
Net histamine release % induced by compound 48/80 + net histamine release % induced by PEI_6	13.5 ± 3.2^D ($n=4$)
Net histamine release % induced by bradykinin and PEI_6	19.2 ± 2.6^E ($n=6$)
Net histamine release % induced by bradykinin + net histamine release % induced by PEI_6	15.6 ± 4.6^F ($n=3$)

Histamine release was measured by the addition of two of the three non-immunologic stimuli simultaneously. The concentrations of compound 48/80, bradykinin and PEI_6 were 0.1 $\mu\text{g/ml}$, 20 μM and 1 $\mu\text{g/ml}$, respectively. Percentages of net histamine release induced by compound 48/80 (0.1 $\mu\text{g/ml}$), bradykinin (20 μM) and PEI_6 (1 $\mu\text{g/ml}$) were 4.5 ± 1.1 ($n=8$), 4.7 ± 1.5 ($n=5$) and 10.7 ± 1.5 ($n=6$), respectively. The spontaneous histamine release was 6.1 ± 0.4 ($n=18$). Differences between A and B , C and D , and E and F were not significant.

residues that were detected by WGA- and PHA- E_4 -lectin blotting, as previously described (17). Inhibition by STA on the histamine release induced by PEI_6 was sugar-specific, but inhibition by PWM on the histamine release induced by PEI_6 was not (Fig. 2B), suggesting that the inhibitory mechanisms of STA and PWM were different. As PWM did not inhibit histamine release induced by compound 48/80 and bradykinin (Fig. 2B), the interaction of PWM with the corresponding glycoproteins was not sufficient to prevent the mast cell activation. Among WGA, STA and PWM, each having an affinity to GlcNAc oligomers, WGA is the most potent inhibitor; its IC_{50} values for inhibition of histamine release induced by compound 48/80, bradykinin and PEI_6 were 160, 41.4 and 37.0 $\mu\text{g/ml}$, respectively, as previously described (17).

Sia is involved in non-immunologic stimuli; neuraminidase-treated rat peritoneal mast cells lost their ability to respond to compound 48/80, substance P, bradykinin and anaphylatoxins (11, 29, 30). Inhibitory effects of MAM on the histamine release induced by compound 48/80, bradykinin and PEI_6 but not that by A23187 (Fig. 1) suggest that binding of MAM to glycoproteins with Sia residues resulted in the inhibition of the histamine release induced by compound 48/80, bradykinin and PEI_6 , but not that by A23187. The inhibitory potency of MAM was more than or almost the same as that of WGA (Fig. 1), suggesting that not only GlcNAc oligomers but also Sia are responsible for the inhibition by WGA. Inhibitory

effects of MAM were consistent with the inhibitory effects of *Limax flavus* agglutinin (LFA), as previously reported (14). It is noteworthy that MAM inhibited the histamine release induced by PEI₆, but SSA did not (Fig. 2A). Although both MAM and SSA have an affinity to Sia, the configurations recognized by the two were different. Sia α 2,3Gal seemed to be more important than Sia α 2,6-Gal.

There are two possibilities to explain the inhibitory effects of MAM and STA. One is that the glycoproteins involved in the inhibition may contain both Sia and GlcNAc residues. Another is that the glycoproteins with Sia are different from the glycoproteins with GlcNAc oligomers. Our preliminary data showed that the inhibitory effect of MAM was not enhanced in the presence of STA, although MAM and STA recognize different sugar residues. The inhibition in the presence of both of MAM and STA was dependent on the inhibitory effect of MAM.

Lectins show mitogenic activities after 2 or 3 days incubation with lymphocytes (31). However, inhibition by lectins on the histamine release was observed in 10 min (Figs. 1–4). Our preliminary data showed that WGA and STA inhibited the histamine release induced by compound 48/80 (0.3 μ g/ml) in 1 min, the % inhibition by WGA (100 μ g/ml) and STA (100 μ g/ml) being 54.7 ± 3.7 ($n=6$, $P<0.001$) and 38.6 ± 3.9 ($n=10$, $P<0.001$) %, respectively. We also observed 45.1 ± 5.0 ($n=6$, $P<0.001$) % inhibition of the histamine release induced by compound 48/80 by the addition of compound 48/80 and WGA simultaneously. It was unlikely that the inhibitory effects depended on cell differentiation by these lectins.

The inhibitory lectins may interfere with the binding of the three non-immunologic stimuli to the specific glycoproteins. The additive effects of the three non-immunologic stimuli suggest that the action sites for compound 48/80, bradykinin and PEI₆ partially overlap each other.

The characteristics of the inhibitory effects of lectins on histamine release reflect the differences between compound 48/80 and bradykinin. PEI₆ resembled bradykinin, except for the inhibitory effect of SSA. Compound 48/80 is, however, different from bradykinin and PEI₆ with respect to its interaction with ABA and PNA (Fig. 3). SJA inhibited the histamine release induced by compound 48/80 (Fig. 4), as described by Bach and Brashler (25). We found that ABA, PNA and SJA were weak inhibitors of histamine release induced by compound 48/80, as was *Ricinus communis* agglutinin (RCA₁₂₀, MW=130,000) (17), suggesting that the mucin-type and asparagine-linked oligosaccharides possessing Gal/GlcNAc residues and Gal β 1,3GalNAc group on the rat mast cell membrane were important for mast cell activation by compound 48/80, but not those by bradykinin and PEI₆.

Compound 48/80, bradykinin and PEI₆ partially shared the signalling pathways including Gi-like G protein. Based on the inhibitory effects of the lectins in this study, we proposed that one of the action sites for compound 48/80, bradykinin and PEI₆ is glycoproteins having asparagine-linked-sugar residues composed of GlcNAc-oligomer, Sia (especially Sia α 2,3Gal) and/or bisecting GlcNAc residues. In addition to Gi-like G proteins, these glycoproteins possibly play important roles in mast cell activation induced by non-immunologic stimuli.

The inhibitory effects of lectins demonstrated in this study were quite different from those effected by the binding of lectins to IgE molecules; in the latter case, lectins bound to sugar residues of IgE molecules instead of mast cell glycoproteins (32). Analyses using lectins revealed that the PGE₁ receptor of mastocytoma P815 cells (33), substance P receptor of porcine brain (34) and M₂-muscarinic receptor of rat (35) were indeed glycoproteins. The lectin-specificities of these receptors differed from the mast cell glycoproteins involved in sugar-specific inhibition by lectins.

It is fortuitous that lectin-specific glycoproteins are one of the action sites for basic secretagogues so that the inhibitory lectins can be utilized to determine these glycoproteins. We are currently examining lectin blotting using a lectin that has affinity to GlcNAc and releases histamine from rat peritoneal mast cells via Gi-like G protein (36). Comparison of glycoproteins specific for the inhibitory lectins with those specific for a lectin that releases histamine will give us useful information to clarify the mechanisms of non-immunologic stimuli via specific glycoproteins on rat peritoneal mast cells. Chemicals that control the functions of the glycoproteins would become helpful therapeutic tools to regulate immediate hypersensitivity.

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