POSSIBLE ROLE OF IgE-CONSTITUENT CARBOHYDRATE IN THE MEDIATION OF HISTAMINE RELEASE

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Abstract—A possible role of IgE-constituent carbohydrate in the mediation of histamine release was pharmacologically studied in isolated peritoneal rat mast cells. Among polysaccharides obtained commercially, mannan and dextran induced histamine release, and the ED50 was 4 µg/ml and 52 µg/ml, respectively. At doses higher than 1 mg/ml, Ficoll, hyaluronic acid and heparin induced the release, while agarose did not. The weak histamine releasing polysaccharides did not induce inhibition of the dextran-induced histamine release. Monosaccharides such as N-acetylglucosamine, mannose and N-acetylneuraminic acid induced marked inhibition of the mannan-induced histamine release, although other carbohydrate constituents of IgE fucose and galactose were weak inhibitors. The antagonism of the monosaccharides against polysaccharide-induced histamine release was found to be a competitive type. Also, antigen-induced histamine release from peritoneal mast cells actively immunized with egg albumin appeared to be inhibited competitively by a carbohydrate constituent of IgE N-acetylglucosamine. Hence it appears that IgE-constituent carbohydrate may play an important role in IgE-mediated histamine release.

It had been reported that mediator release was evoked from mast cells when antigen was reacted with the corresponding IgE bound firmly to the cells (1, 2). This IgE-mediated histamine release may be due to a structural change of IgE molecule bound to mast cells (2, 3). The active center of IgE resulting from the structural change is probably composed of constituent peptides and/or carbohydrates. We attempted to determine whether or not the IgE-constituent carbohydrate is a trigger substance of the histamine release. Carbohydrate composition of human IgE was reported to be N-acetylglucosamine, galactose, mannose, fucose and N-acetylneuraminic acid (4). Polymers composed of the carbohydrates were first studied regarding the stimulating effect on histamine release from isolated rat mast cells. Second, a monosaccharide which was a competitive inhibitor against polysaccharide-induced histamine release was studied as regards the competitive inhibition against IgE-mediated histamine release from rat mast cells.

MATERIALS AND METHODS

Peritoneal mast cells
Male Wistar and Sprague Dawley rats weighing 200–400 g were sacrificed and peritoneal cells were isolated. The mixed cells without purification procedures were kept on ice until use. The concentration of mast cells included was about 8% and about 5% in Wistar and Sprague-Dawley strains, respectively. The suspension medium of peritoneal cells was Tris-
buffered salt solution, pH 7.4, of the following composition: NaCl 121 mM, KCl 4.8 mM, CaCl$_2$ 1.0 mM, tris (hydroxymethyl) aminomethan (Trizma Base, Sigma) 25 mM, bovine albumin (Fraction V, Armour) 0.1 %, HCl for adjustment of pH.

**Release of histamine**

Aliquots of the cell suspension (0.2 ml) were first preincubated at 25°C for 20 min while shaking at 120 Hz. To this suspension 0.2 ml of 30 μg/ml phosphatidyl-L-serine (Nakarai Chemicals, Ltd., Kyoto) and 0.2 ml of polysaccharide solution tested were added and incubation at 25°C was continued for 15 min. The reaction was then stopped by cooling and each tube was centrifuged at 1500 g for 2 min at 0°C. The supernatant was assayed for histamine by fluorometry after condensation with o-phthal-aldehyde according to the method of Shore et al (5) with purification steps omitted. The histamine release was calculated as a percentage of the total histamine content and corrected for spontaneous release in the medium alone. Total histamine content was determined using the supernatant of 0.2 ml aliquot of the peritoneal cell suspension boiled for 2 min together with 0.4 ml of 0.1 N HCl. The value ranged between 2–4 μg.

Inhibitory effects of monosaccharides on histamine release were studied by the simultaneous addition of the test substance and a histamine releaser. When polysaccharides were used as inhibitors, preincubation at 25°C for 15 min was carried out before addition of the histamine releaser. When necessary, the doses of carbohydrates required for 50% inhibition (ID$_{50}$) with 95% confidence limits were calculated.

**Sensitization**

Rats were sensitized with one s.c. administration of 1 mg of crystalized egg albumin (salt-free, grade V, Sigma) and 2 x 10$^8$ cells of *Bordetella pertussis* organisms together with incomplete Freund’s adjuvant. Fourteen to twenty days later, peritoneal mast cells actively sensitized were obtained from these animals.

**Carbohydrates**

All the polysaccharides and monosaccharides used were commercially obtained: Dextran T-2000 and Ficoll (Pharmacia Fine Chemicals), mannan from yeast, colominic acid sodium salt, hyaluronic acid sodium salt, heparin sodium salt, agarose A-37, D-(+)-mannose, D-(+)-galactose, N-acetyl-D-glucosamine, L-(−)-fucose, N-acethylneuraminic acid, methyl α-D-glucoside, D-glucuronic acid, gluconic acid sodium salt and D-(−)-sorbitol (Nakarai Chemicals, Ltd., Kyoto), D-(−)-glucose (Wako Pure Chemical Ind., Ltd., Osaka).

**RESULTS**

**Histamine release induced by polysaccharides**

Polysaccharides were investigated as to the properties which would induce histamine release from peritoneal mast cells of Sprague-Dawley rats, and the results are shown in Fig. 1. Mannan (mannose homopolymer) and dextran (glucose homopolymer) induced histamine release from the peritoneal mast cells and the ED50 was 4 μg/ml, 52 μg/ml, respectively. Ficoll (glucose, fructose polymer), hyaluronic acid (N-acetyl-D-glucosamine, glucuronic
Acid polymer) and heparin (glucosamine 6-sulfate, glucuronic acid 2-sulfate, iduronic acid polymer) induced the release to some extent. Colominic acid (N-acetylneuraminic acid homopolymer), chondroitin sulfate (glucuronic acid, N-acetylgalactosamine polymer), inulin (fructose homopolymer) and agarose (galactose homopolymer) had no such effect.

In Wistar rats, the histamine releasing activity was also studied and the results are shown in Fig. 2. Mannan, dextran and Ficoll induced histamine release from the peritoneal mast cells, although each effectiveness was about 3 fold less than that seen in the Sprague-Dawley rats.

**Failure of histamine release inhibition induced by polysaccharides**

Polysaccharides which possessed little or no effect on histamine release from peritoneal mast cells were investigated to determine whether or not such would inhibit dextran-induced histamine release from peritoneal mast cells of Sprague-Dawley rats. As shown in Table 1, dextran-induced histamine release was not inhibited by any polysaccharide tested at the...
concentration at which the stimulating effect of the polysaccharide alone on histamine release could not be observed. This failure of the inhibition indicated that these polysaccharides had no affinity with the site of releasing action of dextran.

**Histamine release inhibition induced by monosaccharides**

The inhibitory effect of monosaccharides on the histamine release induced by mannan (the most effective releaser among polysaccharides tested) was studied and the results are shown in Table 2. In the IgE-constituent carbohydrates, N-acetyl-D-glucosamine was the most effective inhibitor and D-(-)-galactose was the least effective, indicating that steric conditions of substituent groups in the 2-position and of hydroxy group in the 4-position are required for the inhibitory activity.

Using glucose and its derivatives, the structural requirements for the inhibition were studied. Glucose, methyl α-glucoside and glucuronic acid inhibited to the same extent the mannan-induced histamine release. On the other hand, sorbitol and gluconic acid were

### Table 1. Failure of inhibition induced by polysaccharides against dextran-induced histamine release

| Polysaccharides            | Dose | Relative histamine release |
|----------------------------|------|----------------------------|
| Dextran alone              |      | 100%                       |
| Hyaluronic acid            | 1 mg/ml | 100                        |
| Heparin                    | 1    | 104                        |
| Colominic acid             | 1    | 103                        |
| Agarose                    | 10   | 102                        |
| Chondroitin sulfate        | 10   | 113                        |
| Inulin                     | 10   | 103                        |

Each value represents the mean of 2 experiments. Dextran was used at 1 mg/ml.

### Table 2. Inhibitory effects of IgE-constituent monosaccharides and of glucose and its derivatives on mannan-induced histamine release

| Carbohydrates               | ID50 (95% confidence limits: mM) |
|-----------------------------|----------------------------------|
| N-Acetyl-D-glucosamine      | 1.6 (1.4–2.0)                    |
| N-Acetylneuraminic acid     | 2.7 (2.6–2.9)                    |
| D-(-)-Mannose               | 3.4 (2.2–5.0)                    |
| L-(-)-Fucose                | 5.5 (5.1–6.0)                    |
| D-(-)-Galactose             | 9.2 (7.3–11.4)                   |
| D-(-)-Glucose               | 3.8 (3.2–4.4)                    |
| Methyl α-D-glucoside        | 4.1 (3.3–4.9)                    |
| D-Glucuronic acid           | 4.7 (4.0–5.5)                    |
| D-(-)-Sorbitol              | 19 (15–24)                       |
| Gluconic acid               | >25                              |

Each value was obtained from 4 experiments. Mannan was used at a dose of 30 μg/ml.
weak inhibitors. These observations indicate that an aldehyde group in the 1-position is required for the inhibitory effect on the histamine release induced by mannan.

Mode of inhibitory action of monosaccharides

Modes of inhibitory action of monosaccharides were examined in Wistar and Sprague-Dawley rats. As shown in Fig. 3, the dose-response curve for dextran-induced histamine release from Wistar rat mast cells shifted to the right in the presence of glucose. At the lower doses, the constituent monosaccharide inhibited competitively. Effects of higher doses of the constituent monosaccharide were studied mainly in a mannan-mannose system. The dose-response curve for mannan-induced histamine release from Sprague-Dawley rat mast cells also shifted to the right in the presence of higher doses of mannose as shown in Fig. 4. The inhibition by mannose was overcome markedly but not completely by increasing the dose of mannan. These results indicate that polysaccharide-induced histamine releases are inhibited by the constituent monosaccharide in a competitive type.

![Fig. 3. Mode of inhibitory action of glucose on dextran-induced histamine release from isolated peritoneal mast cells of Wistar rats. Histamine release was expressed as % of histamine release induced by 10 mg/ml dextran. Each point represents the mean of 4–6 experiments and vertical bars represent the s.e. of the mean.](image)

![Fig. 4. Mode of inhibitory action of mannose on mannan-induced histamine release from isolated peritoneal mast cells of SD rat. Histamine release was expressed as % of histamine release induced by 3 mg/ml of dextran. Each point represents the mean of 4–6 experiments and vertical bars represent the s.e. of the mean.](image)
Inhibitory effects of N-acetylglucosamine on IgE-mediated histamine release

N-acetyl-D-glucosamine, the most effective inhibitor of the mannan-induced histamine release and one of the IgE-constituent monosaccharides, was studied to determine whether or not it could competitively inhibit the IgE-mediated histamine release. When peritoneal mast cells harvested from each rat immunized actively with one administration of egg albumin were stimulated to release histamine by incubating with the different doses of antigen, there was an individual variation in the extent of histamine release (Fig. 5). The inhibitory effect of N-acetylglucosamine on the antigen-induced histamine release was studied and the results are shown in Table 3. The monosaccharide inhibited dose-dependently the moderate release reaction, but failed to inhibit the intense reaction.

**DISCUSSION**

The possible role of IgE-constituent carbohydrate in the mediation of histamine release was studied by observing the effects of various polysaccharides and monosaccharides on
crude peritoneal mast cells. The mast cells were used without purification to prevent the influence of carbohydrates. Among the polysaccharides tested, mannan was found to be the most potent releaser. Oligosaccharide units rich in mannose included in Fc portion of IgE was reported by Bennich and von Bahr-Lindström (6) and Baenziger et al (7). The units seem to be a possible active center of the IgE structurally changed by interactions of antigen with IgE. This possibility should be elucidated in studies regarding the effects of oligosaccharides isolated from IgE on the histamine release.

On the other hand, agarose which is built up solely from galactose units induced neither histamine release nor its inhibition. In addition, among IgE constituent monosaccharides, galactose was the least effective inhibitor against mannan-induced histamine release. Galactose units in IgE may not play a role in IgE-mediated histamine release.

All the IgE-constituent monosaccharides used as inhibitors of histamine release were dextro-form except fucose. D-(-)-fucose was less effective than L-(-)-fucose and showed the same activity as D-(-)-galactose. The monosaccharide inhibited not only mannan-induced histamine release but also the antigen-induced histamine release. The mode of inhibitory action on mannan-induced histamine release was of a competitive type. The inhibitory action on weak antigen-induced histamine release was more marked than that on the intense release reaction, indicating a possibility of a competitive type.

From these results, it is suggested that IgE-constituent carbohydrate may play an important role in IgE-mediated histamine release.

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