Anti-allergy Actions of Alkylphenyl α -D-Mannopyranosides

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The inhibitory effects of alkylphenyl α -D-mannopyranosides on histamine release from rat peritoneal mast cells induced by an antigen–antibody reaction were examined. Among the compounds tested, 2,4,6-trimethylphenyl α -D-mannopyranoside exhibited the strongest inhibitory effect. Furthermore, the 2,4,6-trimethylphenyl α -D-mannopyranoside suppressed the Schultz–Dale reaction and 48 h homologous passive cutaneous anaphylaxis (PCA), suggesting that this compound may be a useful lead compound in the development of novel anti-allergy drugs.

Keywords aryl mannopyranoside; antigen–antibody reaction; histamine release inhibition; 2,4,6-trimethylphenyl α -D-mannopyranoside; Schultz–Dale reaction; 48 h PCA

The pathological mechanism involved in type I allergy has been explained as the degranulation of mast cells and basophils, followed by the release of chemical mediators such as histamine, leucotrienes and prostaglandins from these cells.^{1–4)} The degranulation of mast cells occurs in response to immunological stimuli in which the antigen–immunoglobulin E (IgE) antibody reaction on the cell surface predominates. It has been known that the cells involved in immunological responses are activated by a variety of stimuli through the recognition of carbohydrate chains of glycoproteins located at cell surface receptors. IgE has been shown to contain D-mannose-containing carbohydrate chains in the Fc portion of its molecule.⁵⁾ Concanavalin A (Con A), a plant lectin isolated from *Canavalia ensiformis*,⁶⁾ is specifically attached to the carbohydrate chains of IgE to induce the degranulation of the cells and the release of chemical mediators.

The binding site of Con A for saccharides, containing distinct hydrophilic and hydrophobic binding subsites, is located in a deep cavity.⁷⁾ Goldstein and his colleagues studied the structural features of simple sugars and oligosaccharides as inhibitors of the reaction between Con A and dextran and then showed the stereochemical requirements for the interaction between Con A and saccharides.^{8,9)} The stereochemical requirements are that unmodified hydroxyl groups at C-3, C-4, C-6 of the D-mannopyranosyl and glucopyranosyl ring are necessary for binding to Con A, and that the α -configuration at an anomeric carbon atom is preferred. Additionally, Keller reported that high concentrations of mannose and methyl α -D-mannopyranoside markedly suppressed histamine release from rat peritoneal mast cells induced not only by Con A but also by an antigen–antibody reaction.¹⁰⁾ This suggests the existence of the recognition of carbohydrate chains in an antigen–antibody reaction similar to the recognition of Con A of carbohydrate chains in an IgE molecule. We reported that newly synthesized alkylphenyl α -D-mannopyranosides strongly inhibited the histamine release from rat peritoneal mast cells induced by Con A, and in particular, 2,6-dimethyl substitution in the phenyl rings of aglycones markedly enhanced the ability to inhibit.¹¹⁾ From these results, we were interested in determining whether alkylphenyl α -D-mannopyranosides

have anti-allergic actions. In the present studies, we examined the influence of these alkylphenyl α -D-mannopyranosides on histamine release induced by an antigen–antibody reaction. Moreover, the most active inhibitor was tested for its effect on the Schultz–Dale reaction in passively sensitized rat stomach strips and 48 h homologous passive cutaneous anaphylaxis (PCA) in rat.

MATERIALS AND METHODS

Materials Egg albumin, compound 48/80, calcium ionophore A-23187 and phosphatidyl-L-serine (from bovine brain) were purchased from Sigma Chemical Co. *Bordetella pertussis* vaccine was purchased from Wako Pure Chemical Co. The alkylphenyl α -D-mannopyranosides were synthesized in our laboratory according to the method described in the previous paper.¹¹⁾ DSCG was generously supplied by the pharmacology department of this university.

Preparation of Rat Peritoneal Mast Cells Male Wistar rats weighing 200–250 g were exsanguinated and injected intraperitoneally with 20 ml of physiological solution consisting of 137 mM NaCl, 2.7 mM CaCl₂, 1.0 mM MgCl₂·6H₂O, 5.6 mM glucose, 1 unit of heparin/ml, and 5 mM phosphate buffer, pH 7.2. The abdominal region was gently massaged for 2 min and then the peritoneal exudate was collected in a siliconized glass vessel. The cells were purified by a modified version of the method of Németh and Röhllich.¹²⁾ After purification, mast cells of 90% or greater purity were obtained.

Assay of Histamine Release from Passively Sensitized Rat Peritoneal Mast Cells The rat peritoneal mast cells were passively sensitized by 100 μ g/ml of anti-ovalbumin serum according to the method described below. This passively sensitized peritoneal cell suspension (0.7 ml) was mixed with 0.1 ml of a physiological solution containing various concentrations of test substances and 0.1 ml of a physiological solution of phosphatidyl-L-serine (3×10^{-5} g/ml). The mixture was incubated at 37°C for 5 min. The test substances were replaced by physiological solution as controls. The preincubated peritoneal cell suspension with 0.1 ml of egg albumin (antigen) was incubated at 37°C for 10 min. The control was treated without egg albumin in the same manner. The mixture was cooled to 4°C and

centrifuged at $2500 \times g$ at this temperature for 10 min. Histamine in the supernatant and residue were measured, respectively, according to the method of Shore *et al.*¹³⁾ The percent inhibition was calculated as follows:

$$\text{histamine release} = \frac{P_s}{P_s + P_r} \times 100 = A$$

P_s : histamine in supernatant

P_r : residual histamine in cells

$$\text{inhibition (\%)} = 100 - \frac{S - B}{C - B} \times 100$$

S : A obtained from test sample

C : A obtained from control

B : A obtained from blank

Assay of Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80 The peritoneal cell suspension (0.8 ml) was mixed with a test substance dissolved in 0.1 ml of physiological solution. The mixture was incubated at 37°C for 5 min. The test substances were replaced by physiological solution as controls. The preincubated peritoneal cell suspension was mixed with 0.1 ml of compound 48/80 solution (1×10^{-5} g/ml) and incubated at 37°C for 10 min. The physiological solution was added in place of the compound 48/80 solution as a blank. The mixture was cooled to 4°C and centrifuged at $2500 \times g$ at this temperature for 10 min. Histamine in the supernatant and residue was measured. The percent inhibition was calculated as described above.

Assay of Histamine Release from Rat Peritoneal Mast Cells Induced by Calcium Ionophore A-23187 The peritoneal cell suspension (0.89 ml) was mixed with a test substance dissolved in 0.1 ml of physiological solution, and the mixture was then incubated at 37°C for 10 min. The test substances were replaced by physiological solution as controls. The preincubated peritoneal cell suspension was mixed with 0.01 ml of calcium ionophore A-23187 ethanol solution (1×10^{-4} g/ml) and incubated at 37°C for 10 min. Ethanol was added in place of the ethanol solution of calcium ionophore A-23187 as a blank. The mixture was cooled to 4°C and centrifuged at $2500 \times g$ at this temperature for 10 min. Histamine in the supernatant and residue was measured. The percent inhibition was calculated as described above.

Preparation of Anti-ovalbumin Serum The antiserum containing homocytotropic antibody was obtained from rats that were sensitized with egg albumin mixed with heat-killed *Bordetella pertussis* vaccine and aluminium hydroxide gel, according to the method of Stotland *et al.*¹⁴⁾ The antibody titer of the serum was 1:500 as estimated by 48 h homologous PCA.

Effects on Schultz-Dale Reaction in Passively Sensitized Rat Glandular Stomach A strip of rat glandular stomach was passively sensitized by incubation with rat anti-ovalbumin serum (prepared according to the method described above) for 2 h at room temperature. The sensitized strip of rat glandular stomach was suspended in a 10 ml magnus tube (32°C 95% O_2 + 5% CO_2) containing Tyrode solution, and the contractile response was recorded isotonicity. Each test compound was added

to the preparation 5 min before the treatment with antigen (egg albumin, 1×10^{-4} g/ml). The percent contraction was expressed as a percent of the maximal response to serotonin at 1×10^{-6} M.

Effects on 48 h Homologous PCA in Rats The antiserum, prepared according to the method described above, was diluted 10-fold with 0.9% saline. One-tenth ml of the diluted antiserum was injected intradermally into 3 sites on the shaved backs of male Wistar rats weighing 200–250 g. The same volume of 0.9% saline was similarly injected into the opposite side. After 48 h, 0.6 ml of 1% Evans blue solution containing 6 mg of egg albumin was injected intravenously into the tail. Thirty minutes later, the animals were sacrificed by exsanguination and the skins were removed to measure the PCA bluing lesion. The amount of dye was estimated colorimetrically after extraction by the method of Katayama *et al.*¹⁵⁾ The test substances were dissolved in 2% gum arabic and administered intravenously into the tail 30 s prior to a challenge by egg albumin. In the control group, 2% gum arabic was injected intravenously in place of the test substances.

RESULTS AND DISCUSSION

In this laboratory, the anti-allergic effects of a series of compounds were investigated using the inhibitory effects on histamine release induced by $1000 \mu\text{g/ml}$ of ovalbumin as a guide. In the future, we plan to compare these data with the present results. Therefore, we first examined the inhibitory effects of alkylphenyl α -D-mannopyranosides on histamine release from sensitized rat peritoneal mast cells induced by $1000 \mu\text{g/ml}$ of ovalbumin. The results are shown in Table I. The histamine release from sensitized rat mast cells by this concentration of ovalbumin (antigen) was $56.2 \pm 5.7\%$. The spontaneous histamine release was $6.7 \pm 3.8\%$. All of the compounds tested suppressed the histamine release in a concentration-dependent manner. Among the compounds tested, 2,4,6-trimethylphenyl and 4-isopropylphenyl α -D-mannopyranosides showed the strongest activity. Trimethylphenyl and isopropylphenyl compounds were approximately equal in inhibitory ac-

TABLE I. Inhibitory Effects of Alkylphenyl α -D-Mannopyranosides on Histamine Release from Sensitized Rat Peritoneal Mast Cells Induced by an Antigen

Compd.	R	IC ₅₀ (mM)	Compd.	R	IC ₅₀ (mM)
1	H	>100	8	2,6-Dimethyl	2.0
2	4-Methyl	3.6	9	2,4-Dimethyl	2.3
3	3-Methyl	5.0	10	3,4-Dimethyl	2.2
4	2-Methyl	3.7	11	2,3,6-Trimethyl	1.4
5	4-Isopropyl	1.2	12	2,4,6-Trimethyl	0.9
6	3-Isopropyl	1.9	13	2,3,5-Trimethyl	1.7
7	2-Isopropyl	1.7

Fig. 1. Correlation of $-\log IC_{50}$ of Alkylphenyl α -D-Mannopyranosides with a Hydrophobic Substituent Constant (π)

The unit of IC_{50} is mM. The π values were obtained from Hansch and his colleagues.^{16,17)}

tivity, and were more effective than dimethylphenyl and monomethylphenyl compounds. The inhibitory effects of monomethylphenyl compounds were weaker than that of dimethylphenyl compounds, but were far stronger than that of the phenyl compound alone. The differences between the inhibitory effects of the trimethylphenyl compounds were insignificant. A slight difference, found among the other di- and monosubstituted groups too, suggested that the influence of the position of alkyl substituents of the benzene ring on histamine release were small or nonexistent. In addition, these results indicated that the ability of these compounds to inhibit histamine release induced by an antigen may be correlated with the hydrophobicity of the alkyl groups in the phenyl rings of their aglycones. Therefore, to confirm the relationship between their inhibiting potencies and the hydrophobic properties of the substituents, we observed the correlation of the $-\log IC_{50}$ of the compounds tested to their hydrophobic substituent constant (π) values, which have been reported by Hansch and his colleagues.^{16,17)} The result is diagrammed in Fig. 1. The hydrophobic substituent constant (π) of the compounds tested ranged from 0.55–1.65. Within this range, the inhibitory effects on histamine release induced by an antigen–antibody reaction ascended nearly incrementally with the π values, and no cut-off phenomenon of inhibitory activity was observed by these compounds. Moreover, the influence of 2,4,6-trimethylphenyl α -D-mannopyranoside on histamine release induced by various concentrations of ovalbumin was examined. As shown in Fig. 2, the histamine release induced by 1 μ g/ml of ovalbumin was the lowest, but the inhibitory effects on the histamine release induced by this concentration of ovalbumin were the strongest. In addition, the histamine release induced by 10 and 1000 μ g/ml of ovalbumin were almost equal, but the inhibitory effect of the former was about two times stronger than that of the latter. These results suggest that those aryl mannopyranosides inhibit the binding of an antigen to the IgE molecule in a competitive manner. Keller found that histamine release induced by the subsequent addition of Con A and worm allergen was markedly suppressed when the sensitized peritoneal or pleural cells were preincubated with a high concentration of trypsinized monovalent Con A, and thus presumed that the antigenic

Fig. 2. Influences of 2,4,6-Trimethylphenyl α -D-Mannopyranoside (**12**) on Histamine Release Induced by Various Concentrations of Ovalbumin

□, induced by 1 μ g/ml of ovalbumin; ▨, induced by 10 μ g/ml of ovalbumin; ■, induced by 1000 μ g/ml of ovalbumin; □, blank.

determinant in the Fab region of the IgE molecule is similar to the receptor site of Con A.¹⁰⁾ On the basis of this finding, it is suggested that the binding sites for these compounds may be located in the Fab portion of the IgE molecule. Further, the inhibitory effects of 1 mM of 2,4,6-trimethylphenyl α -D-mannopyranoside on histamine release induced by compound 48/80 and calcium ionophore A-23187, releasing the histamine release from mast cells without mediation by an antigen–antibody reaction, were 13 and 10%, respectively. The histamine release induced by compound 48/80 and calcium ionophore A-23187 was 65.7 ± 3.2 and $64.2 \pm 3.2\%$, respectively. In comparison with the antigen–antibody reaction, the inhibitory effects on compound 48/80- and calcium ionophore-induced histamine release were small. These weak inhibitory effects suggested that aryl mannopyranosides inhibit histamine release mainly through inhibiting the binding between antigen and antibody.

In a previous paper,¹¹⁾ we examined the inhibitory effect of compounds **1**–**13** on Con A-induced histamine release and then found that 2,6-disubstituted phenyl α -D-mannopyranosides showed much stronger inhibitory effects than the other regioisomers. Moreover, it is

Fig. 3. Effects of 2,4,6-Trimethylphenyl α -D-Mannopyranoside (**12**) on the Schultz-Dale Reaction in Rat Glandular Stomach

a) Responses to the compounds were expressed as percentage of maximal response to serotonin at 1×10^{-6} M. All values are means $\pm$ S.E.M., $n=3$. b) Significantly different from control at $p < 0.01$ (Student's t -test). c) Significantly different from control at $p < 0.05$ (Student's t -test).

considered that the inhibitory effects of these aryl mannopyranosides on Con A-induced histamine release result from the binding of these compounds to Con A on the basis of the finding that the binding of Con A to D-mannose-containing carbohydrate chains of the IgE molecule,⁵⁾ is believed to be the trigger of Con A-induced histamine release. However, no superior inhibitory action was observed by these 2,6-disubstituted compounds in the antigen-antibody induced histamine release from mast cells. The fact that 2,6-disubstituted phenyl α -D-mannopyranosides did not exhibit any stronger inhibitory effect on histamine release induced by the antigen-antibody reaction than the other regioisomers like those compounds did on Con A-induced histamine release suggests that the binding manner of the Fab moiety of the IgE molecule to carbohydrate chains is different from that of Con A.

The histamine release from mast cells induced by an antigen-IgE antibody reaction is an essential step in the pathological process of a type I allergy.¹⁻⁴⁾ Next, to confirm the anti-allergic action of 2,4,6-trimethylphenyl α -D-mannopyranoside, which exhibited the strongest inhibitory effect on histamine release among the compounds tested, we examined the effect of this compound on the Schultz-Dale reaction in passively sensitized rat glandular stomach tissue. The contraction induced by a specific antigen (egg albumin) was $66.58 \pm 5.80\%$, using the contraction induced by serotonin at 1×10^{-6} M as 100%. As shown in Fig. 3, 2,4,6-trimethylphenyl α -D-mannopyranoside significantly inhibited the contraction of rat glandular stomach tissue induced by an antigen in a concentration-dependent manner. On the other hand, compound **12** exhibited no inhibitory effect on the contraction of the glandular stomach induced by 1×10^{-6} M of serotonin, indicating clearly that the inhibition of the contraction did not result from the direct relaxation of compound **12** on smooth muscle.

TABLE II. Inhibitory Effects of 2,4,6-Trimethylphenyl α -D-Mannopyranoside (**12**) on 48 h Homologous PCA in Rats

Treatment	Dose (mg/kg)	Route	Amount of dye (μ g/site)	Inhibition (%)
Control	—	—	98.9 $\pm$ 10.4	—
12	50	i.v.	102.1 $\pm$ 7.8	-3.24
	100	i.v.	78.6 $\pm$ 7.1	20.53
	200	i.v.	46.9 $\pm$ 5.5	52.58 ^{a)}
DSCG	10	i.v.	20.9 $\pm$ 1.9	78.87 ^{a)}

Each experiment consists of 5–7 observations. a) Significantly different from the control at $p < 0.01$ (Student's t -test).

Furthermore, we proceeded to investigate the effect of 2,4,6-trimethylphenyl α -D-mannopyranoside on 48 h homologous PCA in rat. As shown in Table II, 2,4,6-trimethylphenyl α -D-mannopyranoside at doses of 50, 100 and 200 mg/kg dose-dependently prevented PCA in rats, its effect at 200 mg/kg being significant. Disodium cromoglycate (DSCG), an anti-allergic drug, at a dose of 10 mg/kg also significantly prevented PCA.

From the fact that alkylphenyl α -D-mannopyranosides inhibited the histamine release induced by an antigen-antibody reaction and showed almost no inhibitory effect on the histamine release induced by compound 48/80 or calcium ionophore A-23187, the preventive effects of 2,4,6-trimethylphenyl α -D-mannopyranoside on the Schultz-Dale reaction and on 48 h PCA may be due to the inhibition of an antigen-antibody reaction.

In summary, 2,4,6-trimethylphenyl α -D-mannopyranoside appears to be a useful compound in the development of novel anti-allergy drugs.

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