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Prostaglandin D_2 Generation by Rat Peritoneal Mast Cells Stimulated With *Datura stramonium* Agglutinin and Its Inhibition by Haptenic Sugar and Wheat Germ Agglutinin

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ABSTRACT—The production of prostaglandin D_2 (PGD_2) by rat peritoneal mast cells incubated with *N*-acetyl glucosamine (GlcNAc) oligomer-specific *Datura stramonium* agglutinin (DSA) for 10 min in the presence of 0.3 mM Ca^{2+} was examined. Previously, our group reported that the incubation of rat mast cells with DSA (5 – 100 μg/ml) under similar conditions resulted in a calcium influx and histamine release via a pertussis toxin-sensitive G-protein pathway of the mast cells, and the histamine release was inhibited by haptenic sugar chitooligosaccharides or GlcNAc-specific lectin wheat germ agglutinin (WGA) (K. Matsuda et al., Jpn J Pharmacol **66**, 195 – 204 (1994)). DSA (5 – 100 μg/ml) dose-dependently stimulated the mast cells to generate PGD_2. Chitooligosaccharides (1% w/v) and WGA (100 μg/ml) inhibited the production of PGD_2 induced by 100 μg/ml of DSA, suggesting that the effect of DSA is sugar-specific. A prostaglandin G/H synthase inhibitor NS-398 (*N*-[cyclohexyloxy-4-nitrophenyl] methanesulfonamide) (10 μM) inhibited the formation of PGD_2 induced by DSA (20 μg/ml). These results suggest that the binding of DSA to the corresponding sugar residues on the mast cell surface mediates the signaling of the prostaglandin G/H synthase pathway.

Keywords: Mast cell, Prostaglandin D_2, *Datura stramonium* agglutinin, Wheat germ agglutinin, *N*-Acetyl glucosamine

Connective tissue-type mast cells, such as rat peritoneal mast cell, are activated by two different pathways: an IgE-dependent pathway in which IgE-FcεRI complexes activated tyrosine kinase and *Gab2* (1, 2) and an IgE-independent and pertussis toxin (PT)-sensitive-G-protein pathway (3). Our group reported that a GlcNAc-oligomer-specific *Datura stramonium* agglutinin (DSA) activated rat peritoneal mast cells to induce calcium influx and consequently histamine release in the presence of 0.3 mM Ca^{2+} and the absence of phosphatidylserine (4, 5). The treatment of the cells with chitooligosaccharides (a haptenic sugar of DSA) or PT resulted in the inhibition of the histamine release induced by DSA, suggesting that the mast cell activation induced by DSA was sugar-specific, PT-sensitive, and G-protein-dependent (4). DSA is one of the IgE-independent and PT-sensitive G-protein-dependent stimuli of rat mast cells. GlcNAc-specific wheat germ agglutinin (WGA) did not release histamine under the present study conditions, but it dose-dependently reversed the histamine release induced by DSA, suggesting that WGA acts as a competitive antagonist for the histamine release induced by DSA (4). In the present study, we examined the production of prostaglandin D_2 (PGD_2) induced by DSA in rat peritoneal mast cells and the effects of the haptenic sugar WGA and the prostaglandin G/H synthase inhibitor NS-398 (*N*-[cyclohexyloxy-4-nitrophenyl]methanesulfonamide) (6), on it.

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MATERIALS AND METHODS

Rat mast cells

Peritoneal cells from Sprague-Dawley rats (male, 350 – 400 g) were collected and purified using a Percoll density gradient, as described previously (5). The purified rat mast cells were suspended in HEPES-buffered Tyrode solution containing 0.3 mM $CaCl_2$ (137 mM NaCl, 2.7 mM KCl, 12 mM HEPES, 0.3 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM dextrose and 0.03% bovine serum albumin, pH 7.40). The

purity and viability of the cells in the final preparation were >90% and >95%, respectively, as determined by toluidine blue staining and trypan blue extrusion.

Prostaglandin D_2 generation

The purified mast cells (5×10^5 cells/500 μl) were pre-incubated in the presence and absence of 100 μg/ml WGA, 1% w/v chitooligosaccharides or various concentrations of NS-398 at 37°C for 10 min and then activated by exposure to DSA for 10 min at 37°C. After centrifugation (1,600 × g, 4°C, 10 min), the amounts of PGD_2 in the supernatant were determined. PGD_2 was measured with a PGD_2 [^{3}H]radioimmunoassay (RIA) kit (1 μCi), as described previously (7). Antibody, IgG, is a glycoprotein, but WGA and DSA did not affect the total binding of specific antibody to radioactive PGD_2 in the absence of the rat mast cells, suggesting that the radioactivity after the RIA reflected the amount of PGD_2 generated in mast cells stimulated by DSA. The concentrations of WGA and chitooligosaccharides were similar to those in previous studies of histamine release induced by DSA from rat mast cells by our group (4, 5, 8, 9). Aspirin (100 μM) completely inhibited the generation of PGD_2 from the rat mast cells induced by 100 μg/ml DSA, the amount of PGD_2 generated being <4.6 pg/10^5 cells (n = 3).

Statistical analyses

Statistical significance was evaluated using the unpaired Student's *t*-test with $P = 0.05$ taken as the upper limit of significance.

Chemicals

Datura stramonium agglutinin (DSA: GlcNAc oligomer-specific) and chitoorigosaccharides (mixture of GlcNAc-oligomers) were purchased from Honen Corp. (Tokyo). The prostaglandin G/H synthase inhibitors, aspirin and NS-398 (*N*-[cyclohexyloxy-4-nitrophenyl]methanesulfonamide), were purchased from Sanko Junyaku Co. (Tokyo) and Calbiochem (Darmstadt, Germany), respectively. The PGD_2 [^{3}H]radioimmunoassay (RIA) kit (Amersham TRK890) was from Amersham International plc. (Little Chalfont, UK).

RESULTS

PGD_2 generation by DSA from the rat mast cells

DSA (5 – 100 μg/ml) stimulated the generation of PGD_2 from rat peritoneal mast cells dose-dependently (Fig. 1). The amount of PGD_2 generated by 5, 20 and 100 μg/ml of DSA was 4.6 ± 0.1, 11.5 ± 0.2 and 50.9 ± 2.2 pg/10^5 cells (n = 3), respectively. The effect of DSA was almost diminished by 1% w/v chitooligosaccharides; PGD_2 generated by 20 and 100 μg/ml of DSA in the presence of 1% w/v chitooligosaccharides was 6.2 ± 1.1 (P<0.01 vs PGD_2 generated by 20 μg/ml of DSA, n = 3) and 5.1 ± 0.5 pg /10^5 cells (P<0.001 vs PGD_2 generated by 100 μg/ml of DSA, n = 3), respectively (Fig. 1). There was no difference between PGD_2 generated by 5 μg/ml of DSA in the absence of 1% w/v chitooligosaccharides and that by 100 μg/ml of DSA in the presence of 1% w/v chitooligosaccharides.

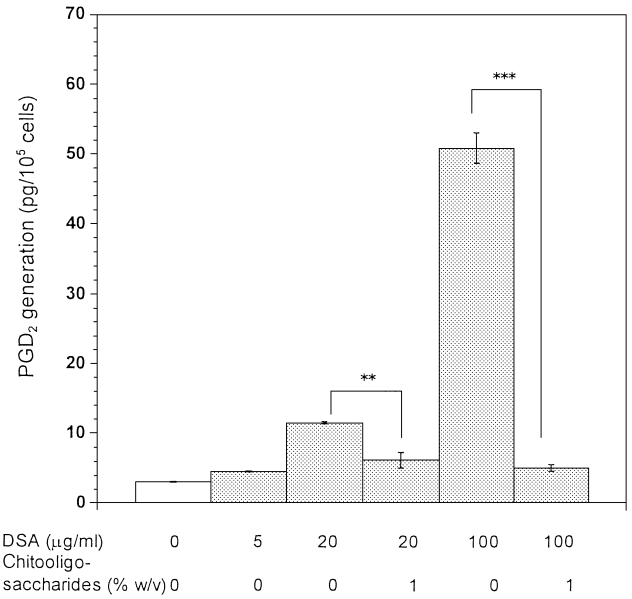

Fig. 1. The production of PGD_2 by mast cells activated with DSA. Purified mast cells were incubated in the presence and absence of chitooligosaccharides (1% w/v) for 10 min at 37°C and then activated by exposure to 5, 20 or 100 μg/ml DSA for a further 10 min. Values are expressed as means ± S.E.M. for 3 experiments. No appreciable increase in PGD_2 was observed in the presence of chitooligosaccharides in the resting mast cells. **P<0.01, ***P<0.001.

Inhibitory effects of WGA on PGD_2 generation from the rat mast cells stimulated by DSA

At a concentration of 100 μg/ml, WGA inhibited the effect of DSA on production. The amount of PGD_2 induced by 100 μg/ml of DSA in the absence and presence of 100 μg/ml of WGA was 50.9 ± 2.2 (control, n = 3) and 4.7 ± 0.5 pg/10^5 cells (P<0.001 vs control, n = 3), respectively (Fig. 2). No difference was observed between the generation of PGD_2 by WGA (100 μg/ml) in the presence and absence of DSA (100 μg/ml).

Effects of NS-398 on the prostanoid generation by mast cells stimulated with DSA

The effects of NS-398 were observed in the presence of 20 μg/ml of DSA. NS-398 dose-dependently inhibited the generation of PGD_2; at the concentrations of 0, 1, 10 and 30 μM, the amount of PGD_2 produced was 24.9 ± 2.5

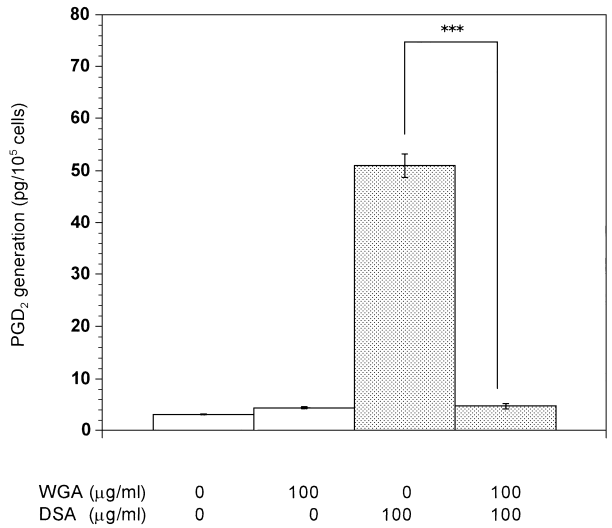

Fig. 2. Inhibition of the production of PGD_2 by WGA in mast cells activated by DSA. Purified mast cells were incubated in the presence and absence of WGA (100 μg/ml) for 10 min at 37°C and then activated by exposure to 100 μg/ml DSA for a further 10 min. Values are expressed as means ± S.E.M. for 3 experiments. ***P<0.001 (The amount of PGD_2 induced by 100 μg/ml of DSA in the absence of 100 μg/ml of WGA vs that by 100 μg/ml of DSA in the presence of 100 μg/ml of WGA).

Table 1. Effect of NS-398 on the production of PGD_2 in mast cells activated by DSA

DSA (μg/ml)	NS-398 (μM)		PGD_2 generation (pg/10^5 cells)
0	0		3.1 ± 0.1
20	0	(control)	24.9 ± 2.5
20	1		20.1 ± 0.2
20	10		12.6 ± 0.8***
20	30		7.9 ± 0.3***

Purified mast cells were incubated in the presence and absence of NS-398 for 10 min at 37°C, and then activated by exposure to 20 μg/ml DSA for a further 10 min. Values are expressed as means ± S.E.M. for 3 experiments. ***P<0.001 vs control.

(control, n = 3), 20.1 ± 0.2 (P>0.05 vs control, n = 3), 12.6 ± 0.8 (P<0.001 vs control, n = 3) and 7.9 ± 0.3 (P<0.001 vs control, n = 3) pg/10^5 cells, respectively (Table 1).

DISCUSSION

DSA is a plant lectin having affinity to GlcNAc-oligomers (10). It dose-dependently and sugar-specifically stimulated the histamine release from rat peritoneal mast cells in the presence of 0.3 mM Ca^{2+} and the absence of phosphatidylserine, as described previously (4). The inhibition by chitooligosaccharides (1% w/v) and WGA (100 μg/ml) on the histamine release induced by DSA (100 μg/ml) was 87.2 ± 3.9% (P<0.001, n = 6) and 32.5 ± 4.0% (P<0.001, n = 4), respectively (4). In the present study, DSA (5 – 100 μg/ml) dose-dependently stimulated the mast cells to generate PGD_2. Chitooligosaccharides of 1% w/v and WGA of 100 μg/ml almost completely inhibited the production of PGD_2 induced by 100 μg/ml of DSA. GlcNAc-specific WGA (100 μg/ml) did not enhance the generation of PGD_2 by DSA (100 μg/ml). WGA did not generate PGD_2 by itself, but inhibited the PGD_2 production by DSA, suggesting that the effect of DSA on the rat mast cells depended on its affinity to GlcNAc-oligomer, but not GlcNAc-monomer.

Activated mast cells produce a variety of chemical mediators: histamine is synthesized and stored in the mast cell granules before activation, whereas prostanoids are synthesized from arachidonic acid after the activation. It was noteworthy that DSA is one of the IgE-independent stimuli of the rat mast cells to release histamine and to generate PGD_2. Chitooligosaccharides and WGA are inhibitors of the histamine release and PGD_2 production. These results suggest that DSA binds the rat peritoneal mast cells via GlcNAc sugar residues to activate PGD_2 generation and WGA binds the corresponding sugar residues of the mast cells to inhibit the binding of DSA. As the mechanisms of inhibition of WGA on the histamine release induced by DSA are competitive, WGA competitively inhibits the PGD_2 production by DSA.

The binding of DSA to the corresponding sugar residues of the mast cells increases the concentration of intracellular calcium ions and releases histamine (5). An increase in intracellular calcium ions consequently appeared to activate phospholipase A_2 (PLA_2) and prostaglandin G/H synthase of the mast cells in addition to the histamine release. To estimate the activities of a PT-sensitive G protein, PLA_2, and prostaglandin G/H synthase will help clarify the signal transduction pathway of the PGD_2 production of the mast cells stimulated by DSA.

In preliminary experiments, NS-398 did not prevent the production of PGD_2 induced by 100 μg/ml of DSA. Therefore, the inhibitory effect of NS-398 on PGD_2 generation was observed at 20 μg/ml instead of 100 μg/ml of DSA. Under these conditions, NS-398 inhibited the PGD_2 production by DSA. We preliminary examined the effect of NS-398 on the histamine release induced by DSA. NS-398 (10 μM) significantly inhibited the PGD_2 production but not the histamine release induced by DSA (20 μg/ml). NS-398 strongly inhibits prostaglandin G/H synthase-2 (COX-2) rather than prostaglandin G/H synthase-1 (COX-1) (6). It was, however, unknown which kind of COX NS-398 inhibited that consequently decreased the PGD_2 generated from rat mast cells induced by DSA. We did not examine the expression of COX-2 in the rat mast cells stimulated by DSA.

Recently, Matsuoka et al. reported that mast cell-derived PGD_2 triggered the receptor of PGD (DP) to induce IgE-dependent asthmatic responses as a mediator to generate T_H2 cytokines IL-4, IL-5 and IL-13 using homozygous mutant ($DP^{-/-}$) mice, suggesting that DP may represent a new therapeutic target for the treatment of allergic asthma (11). It is probable that the mast cell glycoproteins with GlcNAc-residues may be another therapeutic target. Our group reported the mast cell glycoproteins having affinity to DSA detected using lectin-blotting (4). Moreover, our group reported the differentiation of mouse bone marrow-derived mast cells to respond to compound 48/80 (7). These culture mast cells will be useful for examining the glycoproteins having GlcNAc residues coupled to Gi in the signaling pathways responsible for mast cell activation. It remains unanswered whether the IgE-independent activation of mast cells is related to serious asthma and anaphylactic reactions in humans. We are, therefore, interested in the function of the sugar residues recognized by DSA and how they may be involved in mast cell activation.

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