A Peptide Antagonist Derived from Platelet-Derived Growth Factor Induces Histamine Release from Rat Peritoneal Mast Cells

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A synthetic peptide (ANFLVEIIRKKK) designed from the platelet-derived growth factor (PDGF) B-chain, which is known to act as a PDGF antagonist, induced histamine release from rat peritoneal mast cells. Maximal release by the peptide reached about 50% of the total histamine content in mast cells and half-maximal release occurred at 15 μM. The histamine release induced by the PDGF antagonist was required for the presence of Ca2+ in the medium. Treatment of kinase inhibitors (staurosporine and genistein) with mast cells before exposure to the PDGF antagonist inhibited the release to some extent, while calmodulin antagonists (W-7 and R24571) had little effect. The PDGF antagonist induced the secretion of actin from mast cells concurrently with histamine release, though it had no effect on the distribution of tubulin.

These results suggest the possibility that PDGF and its agonists may stimulate and induce exocytosis of peritoneal mast cells.

Key words mast cell; platelet-derived growth factor antagonist; kinase inhibitor; calmodulin inhibitor; histamine release; actin

Mast cells release potent inflammatory mediators such as histamine and serotonin in response to the cross-linking of IgE receptors (FceRI). The stimulation of mast cells is also induced by proteins and peptides including the mast cell degranulating peptide (MCP), melitin, bombesin, adrenocorticotropic (ACTH), mastoparan, substance P, bradykinin, salmine and elupeine. The primary structure of these polypeptides seems to indicate that both continuous basic amino acid residues and neighboring hydrophobic residues play an essential part in their histamine-releasing activity.

Platelet-derived growth factor (PDGF) is a potent mitogen that stimulates the proliferation of a variety of cell types of mesenchymal origin and which may play a role in atherogenesis. This protein has three different dimeric forms, PDGF-AA, -AB, and -BB, which activate two PDGF receptors, α and β. One of the events following the binding of PDGF to its receptor is activation of the intrinsic receptor tyrosine kinase activity and phosphorylation of the receptor on tyrosine residues. PDGF antagonist should be valuable for clinical and laboratory utilities. Synthetic peptides designed based on the primary structure of PDGF have been reported to compete with PDGF binding to both α and β receptors on fibroblasts. More recently, amino acids (Asn116, Phe118, and Leu119) of the PDGF-B chain have been shown to be crucial for the biological activity. We are interested in the properties of PDGF (pI = 9.8) because some active polypeptides that can induce histamine release from mast cells, as mentioned above, involve regions of basic amino acids and of hydrophobic amino acids.

In the present study, we report that a basic peptide (ANFLVEIIRKKK) (pI = 10.6) derived from amino acids 116–121 and 157–163 in the PDGF-B chain, which can compete with PDGF binding to its receptor, induces histamine release from rat peritoneal mast cells. The stimulation is further described with respect to some characteristics and the mechanism.

MATERIALS AND METHODS

Materials PDGF (ANFLVEIIRKKK) antagonist was purchased from Peninsula Lab. Inc. Monoclonal antibody against β-actin was purchased from Amersham. Compound 48/80, staurosporine and R24571 were purchased from Sigma. Genistein, N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide (W-7) and peroxidase-conjugated goat anti-mouse IgG+IgM immunoglobulins were purchased from Wako Pure Chemical Industry.

Mast Cell Preparation and Histamine Release

Mast cells were isolated from male Wistar rats (350–400 g) by centrifugation of peritoneal cells in metrizamide as described; the purity was at least 95% homogeneity. They were suspended at 5 x 10^5 ml^-1 in mast cell medium (MCM) which was comprised of 144 mM NaCl, 2.7 mM KCl, 4.7 mM Na2HPO4, 2 mM KH2PO4, 5.6 mM glucose, 10 U/ml heparin and 1 mg/ml bovine serum albumin (pH 7), then used. The suspension of mast cells (2 x 10^6/tube) was preincubated with or without staurosporine, genistein, W-7 or R24571 at 37°C for 10 min and further incubated with PDGF antagonist or compound 48/80 (0.5 μg/ml) for 10 min at 37°C in a final volume of 0.15 ml. Reactions were terminated by adding 1.85 ml of ice-cold MCM containing 1 mM EGTA and a cooling tube in ice water. After centrifugation, the supernatant was immediately removed and assayed for histamine using o-phthalaldehyde as described by Shore et al. Histamine release from mast cells was calculated by the following equation: [(histamine release)−(spontaneous release)]/(total histamine)−(spontaneous release)) x 100.

Analysis of Actin and Tubulin by Immunoblotting

Mast cells were incubated with 40 μM of the PDGF antagonist and 0.5 μg/ml of compound 48/80 at 37°C for 10 min. At the end of the incubation, mast cells (0.2 ml) were collected by centrifugation at 150 x g for 5 min and mixed with sodium dodecyl sulfate (SDS)-sample buffer (63 mM Tris–HCl, 1% SDS, 5% glycerol, 0.15% β-mercaptoethanol and 0.0001% pyronin Y, pH 6.8), then immediate-

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ly heated to 95°C for 2 min. The supernatants and pellets were analyzed by 10% SDS-polyacrylamide gel electrophoresis (PAGE). Protoplasts separated by SDS-PAGE were electrophoretically transferred to a nitrocellulose filter and excess binding sites on the filters were blocked with 5% skim milk in phosphate buffered saline (PBS) before incubation with the monoclonal antibodies against β-actin and α-tubulin. After incubation with anti-mouse IgG + IgM conjugated with peroxidase, the immunoblot was developed with 4-chloro-1-naphthol as a substrate. The amounts of actin and tubulin were determined by densitometry.

RESULTS AND DISCUSSION

This is the first evidence that a peptide antagonist of PDGF can induce histamine release from rat peritoneal mast cells. The extent of histamine release increased with increasing concentration of the PDGF antagonist added (Fig. 1). In the presence of Ca²⁺, the maximal histamine release reached about 50% of the total histamine content. In contrast, the amount of histamine release was significantly less in the presence of EGTA. The concentration of the peptide required to obtain 50% release was about 15 μM in the presence of 0.5 mM CaCl₂ and 2 x 10⁵ mast cells. This value is in fair agreement with the concentration (26—33 μM) at which the peptide acts as an antagonist of PDGF on human fibroblasts. The action did not change much when heparin, a polyanion, was omitted from the medium, though the histamine-inducing activity by salmine or clupeine, highly basic proteins, was apparently weakened when heparin was added to the reaction medium (data not shown).

Exocytosis from mast cells is known to be triggered by activation of protein kinase activity and/or increase in intracellular Ca²⁺ concentration. Preincubation of staurosporine and genistein, inhibitors of protein serine/threonine kinase and tyrosine kinase, respectively, with mast cells inhibited the histamine-releasing effect of the PDGF antagonist (Fig. 2). This release was decreased to about 70% or 75% of the original level stimulated by the antagonist at 200 nM staurosporine or 20 μM genistein, respectively.

Chakravarty and Nielsen reported that calmodulin, a ubiquitous Ca²⁺-binding protein, is contained in rat mast cells (about 9.4 pmol/10⁶ cells). Calmodulin antagonists, including W-7, chlorpromazine and trifluoperazine, inhibited the mast cell histamine secretion induced by compound 48/80 and Ca²⁺ plus ionophore A-23187. Preincubation of W-7 and R24571 with mast cells had little effect on the release of histamine stimulated by the PDGF antagonist (Fig. 2).

PDGF receptor is also expressed in mast cells. The peptide of PDGF antagonist has been reported to bind to the PDGF receptor and to inhibit its phosphorylation. However, the kinase inhibitors (staurosporine and genistein) suppressed the histamine release from mast cells stimulated by the peptide antagonist, suggesting that the antagonist may act on a target other than the PDGF

Fig. 1. Histamine Release from Mast Cells Induced by the PDGF Antagonist in the Presence and Absence of CaCl₂

Rat peritoneal mast cells (about 2 x 10⁶/tube) were incubated with the indicated concentrations of PDGF antagonist in 150 μl of medium at 37°C for 10 min. The concentrations of CaCl₂ (●) and EGTA (○) were 0.5 and 0.2 mM, respectively. Values are expressed as means ± S.E. of three or four experiments done in duplicate.

Fig. 2. Effects of Kinase Inhibitors and Calmodulin Antagonists on Histamine Release from Mast Cells Induced by PDGF Antagonist

Mast cells were preincubated with the indicated concentrations of drugs at 37°C for 10 min in the presence of 0.5 mM CaCl₂. The cells were further exposed to PDGF antagonist (40 μM) at 37°C for 10 min. P, the PDGF antagonist; S, staurosporine; G, genistein; W, W-7; R, R24571. Values are expressed as means ± S.E. of three or four experiments done in duplicate.

Fig. 3. Analysis of Actin and Tubulin of Mast Cells after Stimulation by SDS-PAGE and Immunoblotting

Immunoblot was performed using anti β-actin (●) and anti α-tubulin (○) monoclonal antibodies. The amounts of proteins of the supernatant (sup) and pellets of mast cells (ppt) were determined from densitometric scans of the sheets. A, non-stimulated mast cells; B, mast cells stimulated by compound 48/80 (0.2 μg/ml); C, mast cells stimulated by PDGF antagonist (40 μM).
receptor. The histamine release by the peptide antagonist was observed in the presence of Ca^{2+} (Fig. 1), but the calmodulin antagonists (W-7 and R24571) had little effect on the peptide antagonist-induced release of histamine from mast cells. These results demonstrate that the effect of the PDGF antagonist may modulate calmodulin-independent phosphorylation which is one of the important pathways of signal transduction for exocytosis from mast cells.

Microfilaments and microtubules have been reported to be responsible for the exocytosis of mast cells. We have recently shown that the actin is also released from permeabilized rat mast cells by Ca^{2+}, and that the release of actin, which is coupled with histamine release, is suppressed by smooth muscle calcium-binding protein 11 (calcinilin or caltropin). We examined the contents of actin and tubulin of mast cells after stimulation by the PDGF antagonist and compound 48/80 using monoclonal antibodies against smooth muscle β-actin and brain α-tubulin (Fig. 3). When mast cells were stimulated by compound 48/80, a small amount of actin was found in the medium, but no detectable tubulin was observed. In contrast, a greater amount of actin was released in the medium after treatment with the PDGF antagonist, though tubulin was not released. These results suggest that the PDGF antagonist is also associated with actin-dependent histamine release from mast cells.

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REFERENCES