A wasp venom mastoparan-induced polyphosphoinositide breakdown in rat peritoneal mast cells

Yukio Okano, Hajime Takagi*, Toyohiko Tohmatsu, Shigeru Nakashima, Yoichiro Kuroda°, Kazuki Saito†, and Yoshinori Nozawa+

Department of Biochemistry, Gifu University School of Medicine, Tsukasamachi-40, Gifu 500, °Department of Neurochemistry, Tokyo Metropolitan Institute for Neurosciences, 2-6 Musashidai, Fuchu, Tokyo 183, and †*Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

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The phospholipid metabolism of rat peritoneal mast cells stimulated with mastoparan, a secretagogue purified from wasp venom, was investigated. Mastoparan at 20 μg/ml caused a rapid secretion of histamine. Mastoparan induced a transient decrease of phosphatidylinositol 4,5-bisphosphate on ^{32}P labeling and generation of a water-soluble degradation product, inositol trisphosphate on [^{3}H]inositol labeling, suggesting the activation of phospholipase C upon stimulation.

Mast cell *Phosphoinositide* *Calcium* *Mastoparan*

1. INTRODUCTION

Rapid progress in understanding the mechanism of intracellular signal transduction has been made. When cell surface receptors are triggered, the signal is transduced to activate phospholipase C hydrolysis of PIP_2, resulting in production of 2 second messengers, DG and IP_3. The former messenger stimulates protein kinase C and the latter mobilizes intracellular Ca^{2+}, both events leading to a variety of cellular responses including secretion, metabolism, phototransduction and cell proliferation [1–3]. As to mast cells, Katakami et al. [4] have reported the activation of protein kinase C in Con A-stimulated cells. Recently, several lines of evidence have indicated the involvement of GTP binding protein in regulating the phospholipase C activity of neutrophils [5] and mast cells [6,7].

Mastoparan is a tetradecapeptide purified from wasp venom and is known to be a potent secretagogue for histamine release from mast cells [8,9]. However, the underlying mechanism of secretion by this peptide has not yet been clarified. Since Higashijima et al. [10] have demonstrated that mastoparan affects the physical state of membranes, it is postulated that this oligopeptide acts as a membrane-perturbing stimulant for mast cells. Argiolas and Pisano [9] have shown that this peptide induces arachidonic acid release in liposomes and rat mast cells, suggesting phospholipase A_2 activation. However, there is no report regarding the effect of mastoparan on polyphosphoinositide metabolism mediated by phospholipase C. Here, we have investigated the phospholipid metabolism of mastoparan-stimulated cells, providing evidence which indicates that a membrane-perturbing

* On leave from the Department of Dermatology, Gifu University School of Medicine
+ To whom correspondence should be addressed

Abbreviations: PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP, inositol phosphate; IP_2, inositol bisphosphate; IP_3, inositol trisphosphate; DG, diacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; GTP, guanosine 5′-triphosphate; MCM, mast cell medium; BSA, bovine serum albumin

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stimulation by mastoparan causes the hydrolysis of PIP_2.

2. MATERIALS AND METHODS

2.1. *Isolation and radiolabeling of rat mast cells*

Mast cells were obtained from the peritoneal cavity of Wistar rats and purified through BSA gradient centrifugation as detailed [11]. The viability of the cells was more than 90% as assessed by toluidine blue staining. Purified mast cells were prelabeled with [^{32}P]orthophosphate (0.5 mCi/10^7 cells) for 60 min at 37°C. Radiolabeled cells were washed with MCM-EGTA to remove free label and resuspended in MCM containing 1.0 mM $CaCl_2$ and 0.5 mM $MgCl_2$. Polyethylene or siliconized glass was used in all experiments.

2.2. *Histamine release*

Mast cells (1–2 × 10^5 cells/0.5 ml) were stimulated with the synthetic peptide, mastoparan. After the designated periods of time, histamine release was terminated by adding an equal volume of 10 mM EDTA-containing MCM [12]. The contents of histamine in the supernatant and pellet were determined by the method of Shore et al. [13] using a Hitachi MPF-3 fluorescence spectrophotometer.

2.3. *Lipid metabolism*

Radiolabeled mast cells (3–5 × 10^5 cells/0.5 ml) were stimulated by exposure to mastoparan at 20 μg/ml for the indicated periods of time at 37°C. For phospholipid analyses, the reaction was terminated by the addition of 2 ml chloroform/methanol (1:2, v/v) and the lipids extracted by the method of Bligh and Dyer [14]. The polyphosphoinositides were extracted with 4 vols chloroform/methanol/conc. HCl (20:40:1, v/v) and separated on HPTLC impregnated with 1% potassium oxalate using the system in [15]. Spots were identified by comigration with authentic standards and radioautography was performed on X-Omat K5 film. The areas corresponding to individual lipids were scraped into vials and the radioactivity determined in a liquid scintillation counter (Beckman LS-7500) with toluene/Triton X-100/water/2,5-diphenyloxazole/2,2′-*p*-phenylenebis(5-phenyloxazole) (2800 ml/700 ml/175 ml/12 g/0.84 g) [16].

2.4. *Inositol phosphate analysis*

Mast cells were labeled with [^{3}H]inositol (37.5 μCi/10^6 cells per 0.1 ml) in Hepes-buffered MCM for 2 h at 37°C under 5% CO_2/air [6]. After radiolabeling, the cells were further incubated in LiCl (10 mM to inhibit inositol-1-phosphatase) containing medium for 30 min at 37°C, then challenged with mastoparan (20 μg/ml) and the reaction was terminated by adding 3.76 vols chloroform/methanol/conc. HCl (50:100:1, v/v) [17]. The aqueous phase was diluted with water, an aliquot of which was employed for total radioactivity counting and the rest was applied on an AG-1 × 8, 100–200 mesh, formate form column (0.6 × 4.0 cm) to elute free inositol. Glycerophosphoinositol, IP, IP_2 and IP_3 were sequentially eluted with 12 ml of 5 mM sodium tetraborate/60 mM ammonium formate, 16 ml of 0.2 M ammonium formate/0.1 M formic acid, 6 × 4 ml of 0.4 M ammonium formate/0.1 M formic acid, and 8 × 2 ml of 1.0 M ammonium formate/0.1 M formic acid, respectively. 2 ml of the IP_3 fraction were first diluted with water to 4 ml, and the radioactivity in 4 ml of each fraction was counted in the gel phase using 60% (v/v) Aquasol-2 [17].

2.5. *Materials*

[^{3}H]Inositol was obtained from Amersham, and [^{32}P]orthophosphate and Aquasol-2 were from New England Nuclear. Compound 48/80 and BSA (fraction V, essentially fatty acid free) were purchased from Sigma, the anion exchange resin AG-1 × 8 was from Bio-Rad, the HPTLC plates were Merck products and the X-Omat K5 film was a Kodak product. All other chemicals were of reagent grade.

3. RESULTS AND DISCUSSION

3.1. *Histamine release from mastoparanstimulated mast cells*

Fig.1A shows a dose-response curve of mastoparan-triggered histamine release from mast cells after 5 min of incubation. The percent histamine release induced by 20 μg/ml mastoparan was 50% and reached 70% at 40 μg/ml. The time course of histamine release shown in fig.1B revealed that the secretory event is completed within 5 s after the addition of mastoparan. A widely

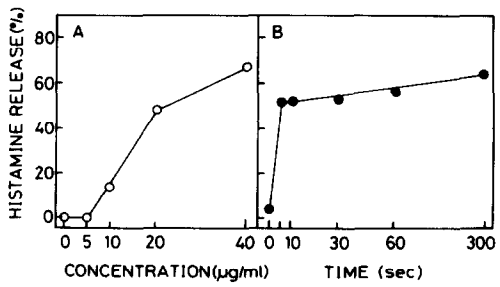

Fig.1. Mastoparan dose-dependent histamine secretion (A) and time course of histamine release (B). Rat peritoneal mast cells were exposed to the indicated concentrations of mastoparan for 5 min at 37°C (A), or to mastoparan at 20 μg/ml at 37°C for the designated periods of time (B). The release reaction was terminated by adding 2 vols of 10 mM EDTA-containing MCM, and the histamine content measured according to Shore et al. [13]. Results are expressed as the percentage of released histamine to the total cell-associated histamine. Each value is the mean of duplicate determination from a representative of 2 similar experiments. Bars indicate the range of 2 determinations.

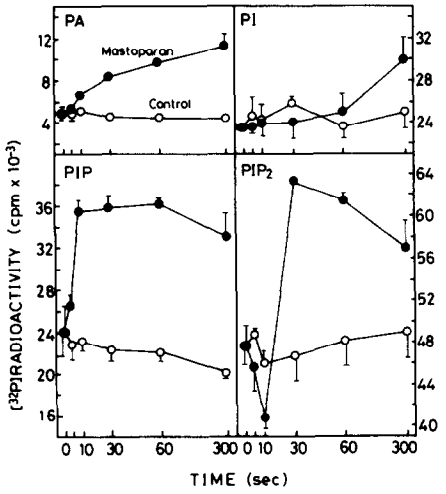

Fig.2. Polyphosphoinositide metabolism in ^{32}P-prelabeled mast cells triggered by mastoparan. Rat peritoneal mast cells, prelabeled with [^{32}P]orthophosphate, were incubated with 20 μg/ml of mastoparan at 37°C for the indicated times. Each value is the mean of duplicate determinations from a representative of 3 similar experiments. Ranges refer to duplicate analysis.

used secretagogue, compound 48/80 at 0.5 μg/ml, induced a slightly higher histamine release (60–70%) than mastoparan (not shown), but the time course of the secretory response was quite similar to that of mastoparan [16].

3.2. *Polyphosphoinositide metabolism of mastoparan-stimulated mast cells*

When [^{32}P]orthophoshate-labeled cells were stimulated with mastoparan (20 μg/ml), the total lipid-associated radioactivity progressively increased up to 140% of the control at 5 min after stimulation (not shown). The radioactivity of PA showed a progressive increase with incubation time. No significant changes in the radioactivity of PI were observed within 60 s following the addition of the oligopeptide. PIP_2 showed a transient decline of radioactivity at 10 s, followed by an abrupt and profound enhancement (fig.2). The PIP_2 breakdown was also ascertained by the generation of IP_3 in [^3H]inositol-labeled cells in accordance with the decrease in [^{32}P]PIP_2 (fig.3). PIP increased from 2.4×10^4 to 3.5×10^4 cpm at 10 s, and did not show any further change in radioactivity within the time period examined (5 min).

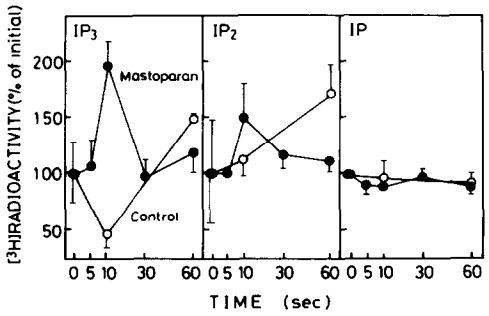

Fig.3. Production of water-soluble inositol phosphates in mast cells stimulated with mastoparan. Rat peritoneal mast cells, prelabeled with [^3H]inositol, were stimulated with mastoparan (20 μg/ml) for the indicated periods of time, and the reaction terminated by adding 3.76 vols chloroform/methanol/conc. HCl (50:100:1, v/v). Water-soluble inositol phosphates were separated by chromatography on anion exchange resin as described in section 2. Results are expressed as percentages of their respective control. The initial radioactivities at zero time for [^3H]IP_3, [^3H]IP_2 and [^3H]IP were 98, 1350 and 2728, respectively. Ranges refer to duplicate analysis.

It has recently been reported that following receptor-ligand coupling, phospholipase C is activated to produce 2 putative second messengers, IP_3 and DG, resulting in cellular responses. The former messenger enhances the level of intracellular free Ca^{2+} and the latter activates protein kinase C. However, the precise mechanism(s) remains to be clarified at the molecular level of activation of membrane phospholipid turnover. As an approach toward the understanding of this mechanism(s), we have attempted to analyze the activated phospholipid metabolism evoked by different types of agonist in mast cells; (i) receptor-mediated (antigen), (ii) by-pass (PMA, ionophore), and (iii) membrane-perturbing (compound 48/80) stimulants. Mastoparan, acting as a secretagogue in mast cells, is known to be intercalated into the lipid bilayer of liposomes [10]. Although mastoparan directly interacts with the membrane, the presence of its receptor has not yet been defined. It is generally accepted that the by-pass stimulants, such as the Ca^{2+} ionophore A23187 and PMA, do not cause PIP_2 hydrolysis. However, the data obtained here, that a membrane-perturbant (mastoparan) elicits the hydrolysis of PIP_2 in response to its exposure, provide evidence that the breakdown of PIP_2 may not be specific for receptor-mediated cell stimulation.

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