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Mastoparan, a peptide toxin from wasp venom, stimulates glycogenolysis mediated by an increase of the cytosolic free Ca^{2+} concentration but not by an increase of cAMP in rat hepatocytes

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A wasp venom, mastoparan, rapidly increased the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) and activated phosphorylase in rat hepatocytes in a concentration-dependent manner. Mastoparan could increase $[Ca^{2+}]_i$ even in the absence of extracellular Ca^{2+}, but a larger increase was observed in the presence of extracellular Ca^{2+}. Thus, mastoparan mobilized Ca^{2+} from intracellular and extracellular Ca^{2+} stores. It also activated inositol triphosphate (IP_3) accumulation, but did not stimulate cAMP production. From these results, we conclude that mastoparan activates rat hepatic glycogenolysis mediated by the accumulation of IP_3, which causes an increase of $[Ca^{2+}]_i$ but not that mediated by cAMP.

Cyclic AMP; Cytosolic free Ca^{2+}; Mastoparan; Phospholipase C; Phosphorylase; (Rat hepatocyte)

1. INTRODUCTION

Mastoparan, a tetradecapeptide purified from wasp venom [1], is a potent stimulator of exocytosis from various endocrine tissues. It can cause secretion of histamine from mast cells, serotonin from platelets, catecholamines from chromaffin cells, and prolactin from the anterior pituitary [2,3]. Okano et al. reported that these effects of mastoparan are mediated by an increase in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) that is triggered with the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by the PIP_2-specific phospholipase C in the plasma membrane [4]. Subsequent studies by Higashijima et al. clarified that the activation of phospholipase C by mastoparan is mediated by direct activation of guanine nucleotide binding protein (G-protein), which is coupled with phospholipase C [5].

Rat hepatic glycogenolysis is known to be activated by adrenergic agonists or glucagon. Epinephrine which acts via an α_1-adrenergic receptor increases $[Ca^{2+}]_i$ and activates hepatic glycogenolysis, while glucagon increases the cAMP content followed by activation of glycogenolysis [6,7]. In both cases, G-proteins are involved in these signal transduction systems, but a different subtype of G-protein is coupled with each effector system, i.e. glucagon receptors are coupled with G_s-protein, which stimulates adenylate cyclase, and α_1-adrenergic receptors are coupled with an unknown G-protein(s) which stimulates phospholipase C [6–8]. In the present study, we compared the effect of mastoparan on $[Ca^{2+}]_i$ and adenylate cyclase in intact liver cells and found evidence that mastoparan activates rat hepatic glycogenolysis mediated by the increase of $[Ca^{2+}]_i$ but not that mediated by cAMP.

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2. MATERIALS AND METHODS

2.1. *Materials*

Slc Wistar rats (9–11 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). Mastoparan was purchased from Peptide Institute (Minoh, Japan) and islet-activating protein, pertussis toxin, was from Kaken Seiyaku K.K. (Kyoto, Japan). L-myo-[^{3}H]inositol (3.02 TBq/mmol) was obtained from Amersham, the anion exchange resin AG 1 × 8 (formate form, 100–200 mesh) was from Bio-Rad, and the cAMP assay kit was from Yamasa Shoyu K.K. (Choshi, Japan). All other chemicals have been described previously [9].

2.2. *Isolation of rat hepatocytes and measurement of $[Ca^{2+}]_i$, phosphorylase activity, and cAMP content*

Rat hepatocytes were isolated from male Slc Wistar rats by the collagenase perfusion method as described elsewhere [9]. Measurements of phosphorylase activity and $[Ca^{2+}]_i$ in rat hepatocytes were also described previously [9]. To measure their cAMP contents, the hepatocytes were preincubated with 0.5 mM isobutylmethylxanthine in Hepes buffer medium (134 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 5 mM $NaHCO_3$, 40 mM glucose, and 10 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethane sulfonic acid, pH 7.4) for 15 min at 37°C under an atmosphere of 95% O_2-5% CO_2 [10]. The preincubated hepatocytes were then incubated with mastoparan for 2 min and the reactions were stopped by the addition of 0.1 N HCl (final concentration). The hepatocytes were homogenized and centrifuged. The resulting supernatant was collected and the cAMP content was measured with a radioimmunoassay kit.

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2.3. *Measurement of inositol phosphate metabolism in hepatocytes*

Hepatocytes were suspended at 3×10^6 cells/ml in Hepes buffer medium containing 0.1% bovine serum albumin and 370 kBq/ml myo-[^{3}H]inositol. Incubations were carried out at 37°C under an atmosphere of 95% O_2-5% CO_2 maintained by continuous gassing for 90 min [11]. The cells were subsequently washed three times to remove excess [^{3}H]inositol. When the inositol phosphate metabolism in the intact hepatocytes was measured, [^{3}H]inositol-labeled hepatocytes were preincubated in Hepes buffer medium containing 10 mM LiCl for 10 min at 37°C, and the reaction was initiated by adding mastoparan. To elucidate the inositol phosphate metabolism in the plasma membrane, prelabeled hepatocytes were homogenized in HEA buffer (50 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid, 1 mM ethylene glycol-bis(*β*-aminoethyl ether) *N,N,N',N'*-tetraacetic acid and 50 kallikrein units/ml aprotinin, pH 7.4) and centrifuged at $1000 \times g$ for 5 min to remove unbroken cells and nuclei. The resulting supernatant was centrifuged at $10000 \times g$ for 15 min, and the resulting pellets were resuspended in HEA buffer (same volume as homogenization) and used as the plasma membrane fractions. This suspension was preincubated with 10 mM LiCl, 6 mM $MgCl_2$, and 500 nM Ca^{2+} at 37°C for 5 min, and the reaction was initiated by the addition of mastoparan and 500 nM of GTP-*γ*-S. After incubation of the plasma membrane for 5 min or for the indicated incubation time in the case of hepatocytes kept at 37°C, the reactions were stopped by adding an equal volume of 15% trichloroacetic acid. The resulting supernatant was washed with diethylether 5 times and neutralized with 50 mM sodium borate. Inositol phosphates in the solution were separated on an AG 1×8 column (formate form) as described previously [12].

2.4. *ADP-ribosylation of liver plasma membranes by IAP*

Rough liver plasma membranes prepared as described in section 2.3 were resuspended in HEA buffer containing 10 mM thymidine and 1 mM NAD at 0.5 mg protein/ml, and ADP ribosylation of this plasma membrane was initiated by addition of 2 *μ*g/ml of activated islet-activating protein (IAP) and incubated at 37°C for 30 min. After the incubation, the membrane suspensions were centrifuged at $10000 \times g$ for 15 min and the pellets were resuspended in HEA buffer. This centrifugation process was repeated 3 times to remove IAP and NAD. No significant [^{32}P]ADP-ribose incorporation into the *α*-subunits of IAP-sensitive G-proteins were observed when the IAP-pretreated liver plasma membrane was ADP-ribosylated by IAP again using [^{32}P]NAD according to the method of Katada [13].

3. RESULTS AND DISCUSSION

Increases in phosphorylase activity and $[Ca^{2+}]_i$ were detected in isolated hepatocytes by the addition of various concentrations of mastoparan, with the patterns indicating that the relationship between these changes and mastoparan concentrations were almost the same (fig.1). We could also observe the glucose output by mastoparan in isolated perfused rat liver (data not shown). The time course changes of the mastoparan-induced increase in $[Ca^{2+}]_i$ were compared with those of epinephrine. As shown in fig.2, epinephrine transiently increased $[Ca^{2+}]_i$, which then rapidly returned to the resting level. With mastoparan, the increase in $[Ca^{2+}]_i$ occurred but no return of the $[Ca^{2+}]_i$ level was observed (fig.2). This result indicated that mastoparan-stimulated intracellular Ca^{2+} mobilization was slightly different from the receptor-operated mechanism and there are two possibilities: (i) mastoparan continuously activates G-protein(s); and (ii) mastoparan cannot activate the Ca^{2+} exclusion

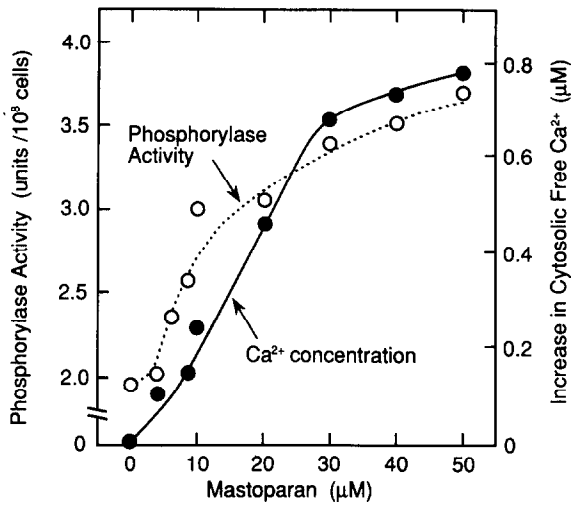

Fig.1. Concentration-dependent enhancement of phosphorylase activity and increase in $[Ca^{2+}]_i$ by mastoparan. Isolated rat hepatocytes were incubated with the indicated concentrations of mastoparan at 37°C, and phosphorylase activities (open circles) were measured 2 min after mastoparan treatment. For the determination of $[Ca^{2+}]_i$, fura-2-loaded hepatocytes were incubated at 37°C with the indicated concentrations of mastoparan and changes of $[Ca^{2+}]_i$ were determined (closed circles). Basal $[Ca^{2+}]_i$ was 294 ± 70 nM (mean ± SE).

system. A mastoparan-stimulated increase of $[Ca^{2+}]_i$ was observed even in the Ca^{2+}-free incubation medium. When Ca^{2+} (final concentration of 1.3 mM) was added to the Ca^{2+}-free incubation buffer after the treatment with mastoparan, a further increase of $[Ca^{2+}]_i$ was detected as shown in fig.3. These results indicate that mastoparan caused a release of Ca^{2+} from the intracellular store and an influx of extracellular Ca^{2+}.

The increase of $[Ca^{2+}]_i$ by receptor activation is known to be triggered by the hydrolysis of PIP_2 in the plasma membrane [14]. Then, we examined the effect of mastoparan on the phosphatidyl inositide metabolism in the rat hepatic plasma membrane. Fig.4

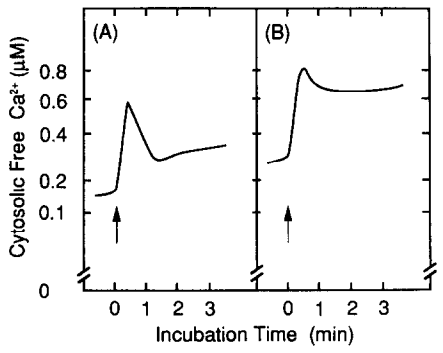

Fig.2. Time course changes of $[Ca^{2+}]_i$ induced by epinephrine (A) or mastoparan (B). Fura-2-loaded rat hepatocytes were incubated at 37°C in the incubation medium containing 1.3 mM Ca^{2+} and the changes of fluorescence intensities were detected after addition of 10 *μ*M epinephrine (A) or 50 *μ*M mastoparan (B). Epinephrine and mastoparan were added at time 0 in the figure.

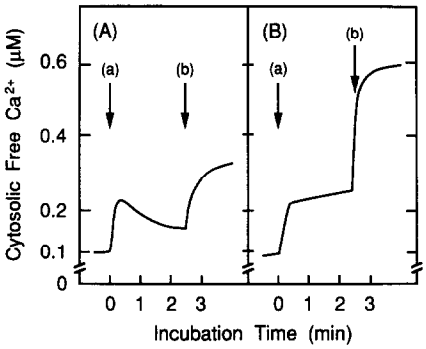

Fig.3. Epinephrine (A) or mastoparan (B) induced an increase in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. Fura-2-loaded hepatocytes were washed out 3 times with Ca^{2+}-free buffer, and incubated in Ca^{2+}-free buffer at 37°C. 10 μM epinephrine (A) or 50 μM mastoparan (B) were added to the incubation medium at time 0 (a) and 1.3 mM $CaCl_2$ (final concentration) was added to the incubation medium at 2.5 min (b).

shows the time course of the accumulation of inositol triphosphate (IP_3), indicating that 50 μM of mastoparan caused IP_3 production, reaching about 8-fold the control level in isolated hepatocytes. The maximal rate of IP_3 production was observed within 1 min and during this period, the increase of $[Ca^{2+}]_i$ was also maximal (fig.2). IP_3 production by mastoparan was also observed in a cell-free system (fig.5). These results show that mastoparan stimulated the increase of $[Ca^{2+}]_i$ associated with PIP_2 hydrolysis in liver plasma membrane followed by activation of hepatic glycogenolysis.

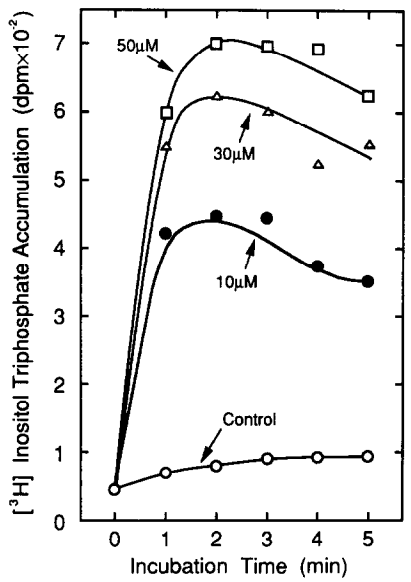

Fig.4. Mastoparan-stimulated IP_3 production. Isolated rat hepatocytes, prelabeled with [^{3}H]inositol as described in section 2, were incubated with mastoparan for the indicated periods of time, and the reaction was terminated by adding an equal volume of trichloroacetic acid. Inositol phosphates were separated and detected as described in section 2.

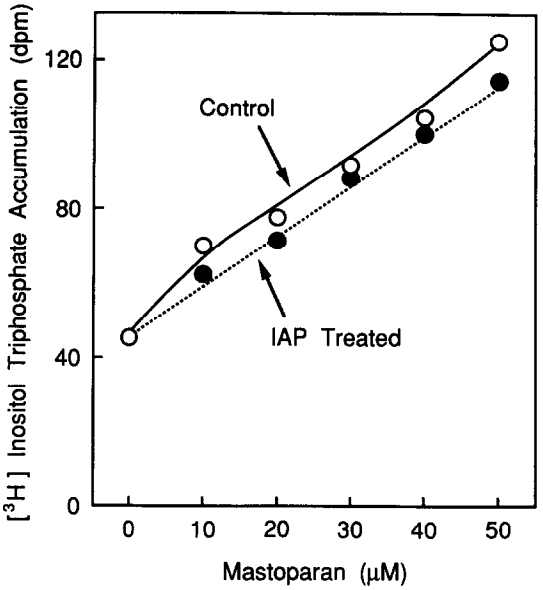

Fig.5. Effect of IAP on mastoparan-stimulated IP_3 accumulation. Rat liver plasma membranes prepared from [^{3}H]inositol-prelabeled hepatocytes were treated with (closed circles) or without (open circles) IAP as described in section 2. The plasma membranes were incubated with 500 nM GTP-γ-S, 500 nM Ca^{2+} and the indicated concentrations of mastoparan for 5 min at 37°C. IP_3 was measured as given in fig.4.

Higashijima et al. showed that mastoparan stimulated the steady state level of GTP hydrolysis by G-proteins and this effect had a selectivity for different types of G-proteins using purified G-proteins [5]. In rat hepatocytes, the α_1-adrenergic receptor is coupled with unknown G-protein(s) which stimulates PIP_2-specific phospholipase C, while the glucagon receptor is coupled with G_s-protein which stimulates adenylate cyclase [6–8]. Therefore, we compared the effect of mastoparan on G_s protein and G-protein coupled with phospholipase C in intact rat hepatocytes. Although 50 μM of mastoparan and 1 nM of glucagon stimulated phosphorylase activity at the same level, an increase in cAMP was observed only on addition of glucagon (table 1). This finding indicates that mastoparan could

Table 1

Effects of mastoparan or glucagon on phosphorylase activity and cAMP accumulation

Treatment	Phosphorylase activity (units/10^8 cells)	cAMP accumulation (pmol/10^5 cells)
Control	1.97	3.67
Mastoparan (50 μM)	3.71	4.13
Glucagon (1 nM)	3.43	7.34
Glucagon (1 μM)	6.24	82.5

Isolated rat hepatocytes were incubated with 50 μM mastoparan and 1 nM or 1 μM glucagon for 2 min at 37°C. When measuring cAMP content, 0.5 mM isobutylmethylxanthine was added to the incubation buffer. The phosphorylase activity and cAMP content in the hepatocytes were measured as described in section 2.

not stimulate G_s-protein, although it probably acts on the phospholipase C-coupled G-proteins. Higashijima et al. also suggested that IAP, pertussis toxin, inhibits the mastoparan-stimulated GTP-γ-S binding activity of G-proteins [5]. And so, the effect of IAP on mastoparan stimulating IP_3 accumulation in liver plasma membranes was then examined to obtain further information about the type of G-protein (fig.5). Mastoparan stimulated IP_3 accumulation in the plasma membrane in a concentration-dependent manner, but IAP did not inhibit mastoparan-stimulated IP_3 accumulation. We recently observed that G-protein(s) coupled with α_1-adrenergic receptors is IAP sensitive (manuscript in preparation). We do not know why the sensitivity of IAP differs between α_1-adrenergic receptor-coupled G-protein and mastoparan-sensitive G-protein(s), but speculate that it may be caused by IAP not being able to completely inhibit the GTP-γ-S binding activity of G-protein [5]. Another possibility is the existence of different G-proteins which stimulate PIP_2 hydrolysis but have different affinities for mastoparan. Fitzgerald et al. and Aiyar et al. have reported that liver G-protein coupled with vasopressin receptors is IAP insensitive [15,16], which offers support to the above hypothesis.

The present findings indicate that mastoparan can activate specific G-proteins in liver cells coupled with PIP_2-specific phospholipase C and increase $[Ca^{2+}]_i$ resulting in activation of the phosphorylase. This effect of mastoparan is not inhibited by IAP.

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