

Mastoparan inhibits phosphoinositide hydrolysis via pertussis toxin-intensive G-protein in human astrocytoma cells

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Mastoparan inhibited [³H]inositol phosphate accumulation induced by carbachol as well as cyclic AMP accumulation induced by isoproterenol in 1321N1 human astrocytoma cells. Mastoparan inhibited GTP γ S-induced, but not Ca²⁺-induced, [³H]inositol phosphate accumulation in membrane preparations with an IC₅₀ of approximately 10 μ M. The inhibitory effect of mastoparan on carbachol-induced [³H]inositol phosphate accumulation was resistant to pertussis toxin (IAP) treatment in intact cells. These results suggest that mastoparan inhibits phospholipase C in human astrocytoma cells via a GTP binding protein, which is not a substrate for IAP.

Mastoparan; Phospholipase C; GTP binding protein; Pertussis toxin; Inositol phosphate

1. INTRODUCTION

Mastoparan is a wasp venom toxin with a structure of Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH₂, which has several pharmacological effects, such as secretion of histamine from mast cells, secretion of serotonin from platelets and secretion of catecholamines from chromaffin cells [1,2]. Histamine release from mast cells by mastoparan has been investigated in detail. Mastoparan stimulates hydrolysis of phosphatidylinositol bisphosphate in mast cells [3], producing two second messengers, inositol trisphosphate [4] and diacylglycerol [5]. The release of histamine by mastoparan was inhibited by IAP, indicating that a G-protein which is a substrate for IAP was involved in mastoparan-induced activation of histamine release [6]. Recently, Higashijima et al. [7] reported that mastoparan increased the binding of [³⁵S]GTP γ S to purified G_o and/or G_i in phospholipid vesicles and also stimulated GTPase activities of G_o. Increase in the [³⁵S]GTP γ S binding to G_o by mastoparan was partially attenuated by IAP treatment. The data suggest that mastoparan might interact with an IAP-sensitive G-protein directly.

Human astrocytoma cells (1321N1) proved to be a useful cell line to investigate receptor-mediated trans-

membrane control. The cells have adrenergic β -receptors [8], A₁-adenosine receptors [9], muscarinic receptors [10], H₁-histamine receptors [11], bradykinin receptors [12] and thromboxane A₂ receptors [13]. Phosphoinositide hydrolysis induced by stimulations of muscarinic, H₁-histamine, bradykinin and thromboxane A₂ receptors is dependent on GTP, but resistant to IAP treatment [11,13–15], meaning that a G-protein involved in phospholipase C activation is not a substrate for IAP in human astrocytoma cells.

In the present study, the effect of mastoparan on phosphoinositide hydrolysis was examined in human astrocytoma cells. The results suggest that mastoparan potentially inhibits phosphoinositide hydrolysis in these cells.

2. MATERIALS AND METHODS

2.1. Materials

Fetal bovine serum was obtained from Cell Culture Laboratory (Cleveland, OH, USA). Aluminum oxide (neutral) was from Merck. Mastoparan was from Peptide Institute (Osaka, Japan). IAP was from Funakoshi Pharmaceutical (Tokyo, Japan). [2-³H]Myo-inositol was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). The sources of other reagents were those described in [13].

2.2. Cell culture

Human astrocytoma cells (1321N1) were grown on 150-mm culture dishes in DMEM containing 5% fetal bovine serum, 50 U/ml of penicillin and 50 μ g/ml of streptomycin. Cells were maintained in a 37°C humidified incubator in an atmosphere of 95% air and 5% CO₂.

2.3. Determination of cyclic AMP

Cells were grown on 35 mm or 12-well plates at a density of 10⁵ cells/ml after subculture with trypsin. Cells were used 4 days after the

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Abbreviations: IAP, islet activating protein (pertussis toxin); GTP γ S, guanosine 5'-[γ -thio]triphosphate; G-protein, guanine nucleotide regulatory protein; cyclic AMP, adenosine 3',5'-monophosphate; DMEM, Dulbecco's modified Eagle's medium; EMEM, Eagle's minimum essential medium

subculture. Incubation was started by addition of drugs in EMEM-Hepes (pH 7.35) after several washes of cells with EMEM-Hepes (pH 7.35). The reaction was terminated by addition of 1 ml of 5% TCA after aspiration of the medium. The TCA-extracted materials were applied to a 1 ml column with a bed volume of aluminum oxide (neutral). Cyclic AMP was eluted by 3 ml of 0.5 M Tris-HCl (pH 7.4) after washing the column with 4 ml of water. Cyclic AMP was determined by a binding-protein method, described previously [16].

2.4. Assay of [^3H]inositol phosphates in intact cells

Phosphoinositide breakdown was monitored by measuring [^3H]inositol phosphates as previously described [11,13]. Cells were grown on 35-mm dishes or 12-well plates at a density of 10^5 cells/ml after subculture. The monolayers were labeled with DMEM containing [^3H]inositol (1 $\mu\text{Ci}/\text{ml}$). Incubation was started by the addition of drugs to EMEM-Hepes (pH 7.35) containing 10 mM LiCl, and was terminated by the addition of 1 ml of 5% TCA. After removing TCA by ether washes, total inositol phosphates were separated by an anion-exchange column (AG 1X-8).

2.5. Assay of [^3H]inositol phosphates in membrane preparations

The preparation of membranes and the analysis of [^3H]inositol phosphates in the membrane preparations were done with essentially the same method as previously described [13,17]. Membranes (50–100 μg) containing [^3H]phosphoinositides (30000–85000 dpm) were used for one assay.

2.6. Data analysis

The statistical significance of the difference between the values obtained was determined with the paired Student's *t*-test.

3. RESULTS

Isoproterenol (10 μM)-activated adenylate cyclase, resulted in an accumulation of cyclic AMP in 1321N1 human astrocytoma cells (fig.1). Mastoparan (30 μM) inhibited the accumulation of cyclic AMP induced by isoproterenol, supporting the observation that mastoparan could directly activate G_i/G_o reported by Higashijima et al. [7]. In addition to inhibition of cyclic

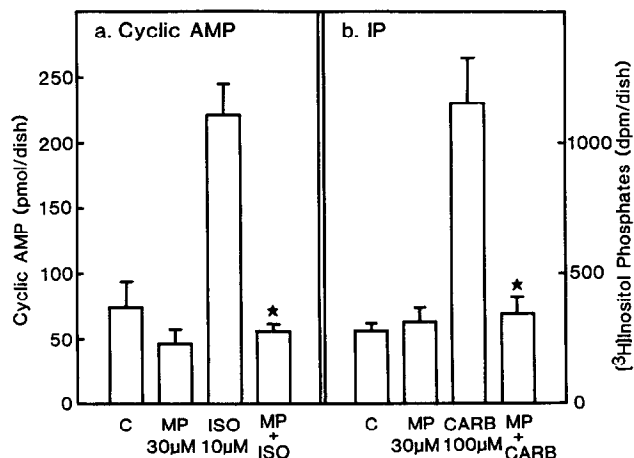


Fig.1. Effect of mastoparan on cyclic AMP accumulation and inositol phosphate accumulation. (a) Mastoparan (30 μM , MP) significantly inhibited isoproterenol (10 μM , ISO)-induced cyclic AMP accumulation (* $P < 0.05$). (b) MP (30 μM) significantly inhibited carbachol (100 μM , CARB)-induced inositol phosphate accumulation (* $P < 0.05$). C, without drug. Results were mean \pm SE of three determinations.

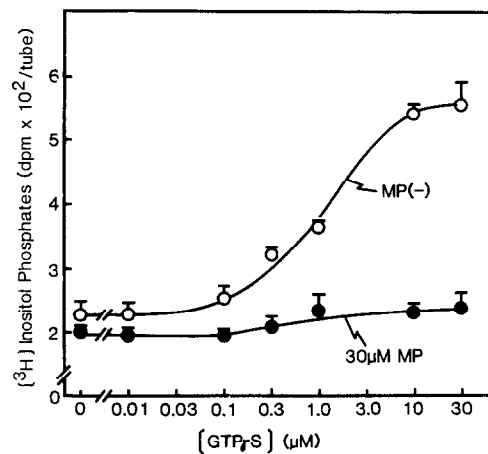


Fig.2. Dose-response curve for GTP γ S-induced accumulation of inositol phosphates in the presence (●—●) or absence (○—○) of 30 μM mastoparan (MP) in membrane preparations. Membranes containing [^3H]phosphoinositides (31000 dpm) were used for one assay. Results were mean \pm SE of three determinations.

AMP accumulation, mastoparan (30 μM) also inhibited phosphoinositide hydrolysis induced by carbachol (100 μM) in intact cells. Since there is no report that an activation of G_i inhibits phosphoinositide hydrolysis, the mechanism of inhibition of phosphoinositide hydrolysis by mastoparan might be different from an activation of G_i .

To elucidate the inhibitory mechanism of mastoparan, we used membrane preparations in which G-proteins were directly activated. GTP γ S (0.1–30 μM) increased inositol phosphate accumulation in a concentration-dependent manner (fig.2). GTP γ S-induced inositol phosphate accumulation was potently inhibited by 30 μM mastoparan (fig.2). The inhibitory effect of mastoparan on GTP γ S (10 μM)-induced ino-

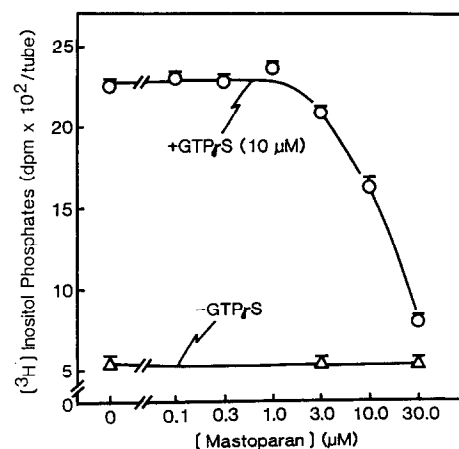


Fig.3. Effect of mastoparan on GTP γ S-induced inositol phosphate accumulation in membrane preparations. Mastoparan inhibited GTP γ S (10 μM)-induced inositol phosphate accumulation (○—○), but did not affect the basal inositol phosphates (Δ — Δ). Membranes containing [^3H]phosphoinositides (81000 dpm) were used for one assay. Results were mean \pm SE of three determinations.

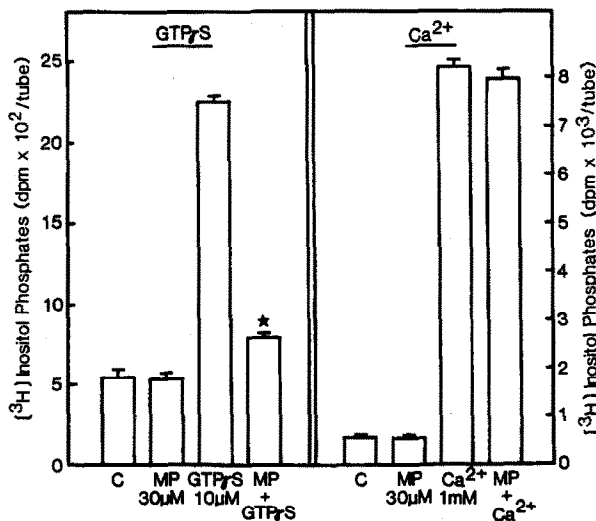


Fig.4. Effect of mastoparan (MP) on GTP γ S- and Ca²⁺-induced accumulations of inositol phosphates in membrane preparations. MP significantly inhibited GTP γ S (10 μ M)-induced inositol phosphate accumulation (* $P < 0.05$), but not Ca²⁺ (1 mM)-induced accumulation. C, without drug. Membranes containing [³H]phosphoinositides (83000 dpm) were used for one assay. Results were mean \pm SE of three determinations.

sitol phosphate accumulation was also dependent on the concentrations used (fig.3). Ca²⁺ ions are known to activate phospholipase C directly in millimolar concentrations [15]. Mastoparan (30 μ M) inhibited GTP γ S (10 μ M)-induced inositol phosphate accumulation, but not Ca²⁺ (1 mM)-induced accumulation (fig.4), indicating that mastoparan directly inhibited the activation of the G-protein.

Pretreatment of astrocytoma cells with IAP (100 ng/ml) for 48 h elicited ADP-ribosylation of endogenous G_i [11,13], resulting in the inhibition of the receptor-G_i/G_o-mediated response [9]. However, the

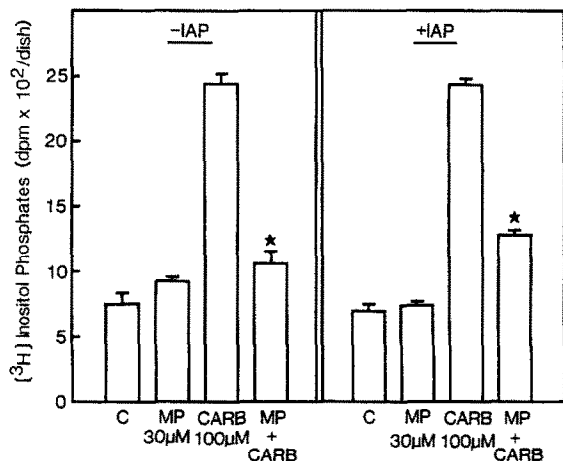


Fig.5. Effect of IAP on mastoparan (MP) inhibition of carbachol (CARB)-induced accumulation of inositol phosphates. The cells were treated with or without 100 ng/ml of IAP for 48 h. MP significantly inhibited CARB-induced inositol phosphate accumulation (* $P < 0.05$) with or without IAP treatment. C, without drug. Results were mean \pm SE of three determinations.

inhibitory effect of mastoparan on inositol phosphate accumulation induced by carbachol was resistant to treatment of the cells with IAP (fig.5), indicating that mastoparan inhibited a putative G-protein which activates phospholipase C and is insensitive to IAP.

4. DISCUSSION

The present study demonstrates that mastoparan inhibits phosphoinositide hydrolysis in intact astrocytoma cells as well as in the membrane preparations derived from these cells. Since mastoparan potently inhibited GTP γ S-induced phosphoinositide hydrolysis, but not Ca²⁺-induced hydrolysis in membrane preparations, mastoparan might inhibit a putative G-protein to stimulate phospholipase C. Recently, Wojcikiewica and Nahorski [18] demonstrated that mastoparan inhibited phosphoinositide hydrolysis induced by GTP γ S in permeabilized SH-SY5Y human neuroblastoma cells. They proposed a possibility that mastoparan interacts with polyphosphoinositides to inhibit their hydrolysis. In fact, mastoparan potently interacts with phospholipids [19]. However, mastoparan stimulates phosphoinositide hydrolysis in mast cells, possibly by activating an IAP-sensitive G-protein [6]. The interaction of mastoparan with polyphosphoinositides alone could not explain the mastoparan-induced hydrolysis of polyphosphoinositides in mast cells. Moreover, the fact that the inhibitory effect of mastoparan on Ca²⁺-induced phosphoinositide hydrolysis was not observed in human astrocytoma cells, and was extremely weaker in SH-SY5Y cells [18], indicates that mastoparan could inhibit a putative G-protein resulting in the inhibition of phosphoinositide hydrolysis.

There are at least two G-proteins to activate phospholipase C, IAP-sensitive [20,21] and IAP-insensitive [11,13-15]. It is interesting that mastoparan activates an IAP-sensitive G-protein to activate phospholipase C in mast cells and it inhibits an IAP-insensitive one in astrocytoma cells. Discrimination of phospholipase C activation by mastoparan in different cell types suggests that there are at least two mechanisms of the G-protein-mediated activation of phospholipase C.

In conclusion, mastoparan inhibits the G-protein which couples to phospholipase C in human astrocytoma cells, and mastoparan could be an important toxin to analyze the mechanism of G-protein-mediated activations of phospholipase C.

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REFERENCES

- [1] Hirai, Y., Yasuhara, T., Yoshida, H., Nakajima, T., Fujino, M. and Kitada, C. (1979) Chem. Pharm. Bull. (Tokyo) 27, 1942-1944.

- [2] Kuroda, Y., Yoshioka, M., Kumakura, K., Kobayashi, K. and Nakajima, T. (1980) *Proc. Jpn. Acad.* 56, 660-664.
- [3] Okano, Y.T., Takagi, H., Tohmatsu, T., Nakashima, S., Kuroda, Y., Saito, K. and Nozawa, Y. (1985) *FEBS Lett.* 188, 363-366.
- [4] Berridge, M.J. and Irvine, R.F. (1984) *Nature (Lond.)* 312, 315-321.
- [5] Nishizuka, Y. (1984) *Nature (Lond.)* 308, 693-698.
- [6] Higashijima, T., Uzu, S., Nakajima, T. and Miyazawa, T. (1987) in: *Peptide Chemistry (Miyazawa, T. ed.)* pp.75-78, Protein Research Foundation, Osaka, Japan.
- [7] Higashijima, T., Uzu, S., Nakajima, T. and Ross, E.M. (1988) *J. Biol. Chem.* 263, 6491-6494.
- [8] Harden, T.K., Cotton, C.U., Waldo, G.L., Lutton, J.K. and Perkins, J.P. (1980) *Science* 210, 441-443.
- [9] Hughes, A.R. and Harden, T.K. (1986) *J. Pharmacol. Exp. Ther.* 237, 173-178.
- [10] Meeker, R.B. and Harden, T.K. (1982) *Mol. Pharmacol.* 22, 310-319.
- [11] Nakahata, N., Martin, M.W., Hughes, A.R., Hepler, J.R. and Harden, T.K. (1986) *Mol. Pharmacol.* 29, 188-195.
- [12] Orellana, S., Salski, P.A. and Brown, J.H. (1987) *J. Biol. Chem.* 262, 1638-1643.
- [13] Nakahata, N., Matsuoka, I., Ono, T. and Nakanishi, H. (1989) *Eur. J. Pharmacol.* 162, 407-417.
- [14] Masters, B.B., Martin, M.W., Harden, T.K. and Brown, J.H. (1985) *Biochem. J.* 227, 933-937.
- [15] Hepler, J.R. and Harden, T.K. (1986) *Biochem. J.* 239, 141-146.
- [16] Nakahata, N., Nakanishi, H. and Suzuki, T. (1977) *Jpn. J. Pharmacol.* 27, 341-349.
- [17] Nakahata, N., Matsuoka, I., Suzuki, T. and Nakanishi, H. (1989) *Res. Commun. Chem. Pathol. Pharmacol.* 64, 179-192.
- [18] Wojcikiewica, R.J.H. and Nahorski, S.R. (1989) *FEBS Lett.* 247, 341-344.
- [19] Higashijima, T., Wakamatsu, K., Takemitsu, M., Fujino, M., Nakajima, T. and Miyazawa, T. (1983) *FEBS Lett.* 152, 227-230.
- [20] Nakamura, T. and Ui, M. (1985) *J. Biol. Chem.* 260, 3584-3593.
- [21] Ohta, H., Okajima, F. and Ui, M. (1985) *J. Biol. Chem.* 260, 15771-15780.