Mastoparan, a Peptide Toxin from Wasp Venom, Mimics Receptors by Activating GTP-binding Regulatory Proteins (G Proteins)*

(Received for publication, January 11, 1988)

Tutomo Higashijima, Sonoko Uzui, Terumi Nakajima, and Elliott M. Rose

From the Department of Pharmacology, Southwestern Graduate School of Biomedical Sciences, University of Texas Health Science Center, Dallas, Texas 75235-9041 and the Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Tokyo 113, Japan

Mastoparan, a peptide toxin from wasp venom, is a nonspecific secretagogue. We show here that mastoparan increases the GTPase activity and the rate of nucleotide binding of several purified GTP-binding regulatory proteins (G proteins) whose function is to couple cell-surface receptors to intracellular mediators. Mastoparan accelerated guanosine-5′-(3′-O-thiotriphosphate binding and consequent G protein activation in part by promoting the dissociation of bound GDP, the mechanism by which receptors regulate G proteins. ADP-ribosylation by pertussis toxin, which uncouples receptors from G proteins, selectively inhibited mastoparan-stimulated activation. Like receptors, mastoparan was more potent if the G protein was reconstituted in phospholipid vesicles and was active at micromolar concentrations of Mg2+. The structure of mastoparan in a lipid bilayer is similar to that predicted for a cationic intracellular loop of G protein-coupled receptors. Mastoparan thus displays a novel mode of toxicity by acting directly on G proteins to mimic the role normally played by agonist-ligated receptors.

Mastoparan, a toxin from wasp venom with the structure Ile-Asn-Leu-Lys-Ala-Leu-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH₂ (7) is a potent stimulator of exocytosis from diverse mammalian cells. It causes secretion of histamine from mast cells, serotonin from platelets, catecholamines from chromaffin cells, and prolactin from the anterior pituitary (1-3). In the case of histamine secretion, the effect of mastoparan is mediated by an increase in cytoplasmic Ca2+ that is itself caused by an increase in the intracellular second messenger inositol-1,4,5-triphosphate (2P3)'.

Inositol-1,4,5-triphosphate; PS, phosphatidylserine; PE, phosphatidylethanolamine.

This work was supported by National Institutes of Health Grant GM30355 and R. A. Welch Foundation Grant 1-982. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: IP₃, inositol-1,3,5-triphosphate; G, G, and G (transducin), GTP-binding regulatory proteins; GTPγS, guanosine-5′-(3′-O-thiotriphosphate); PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine.

* This work was supported by National Institutes of Health Grant GM30355 and R. A. Welch Foundation Grant 1-982. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** S. Uzui, T. Nakajima, T. Miyazawa, and T. Higashijima, manuscript in preparation.

**S. Uzui, T. Nakajima, T. Miyazawa, and T. Higashijima, manuscript in preparation.

EXPERIMENTAL PROCEDURES

G, and G, were purified from rabbit liver by the method of Bokoch et al. (11) and G, was purified from bovine brain by the method of Sternweis and Rohlish (12). Transducin was eluted with GTP from bovine disc membranes (13) and further purified by chromatography on DEAE-Sephacel in 20 mM NaHEPES (pH 8.0), 1 mM EDTA, 1 mM dithiothreitol using a 25-250 mM gradient of NaCl. Mastoparan was synthesized and purified as described (14). Lipids were purchased from Avanti Polar Lipids, pertussis toxin from List Biologicals, and other reagents from sources listed previously (15, 16). Methods for the assay of GTPγS binding, GDP release, and GTP hydrolysis have been described previously (15, 16). All reactions were carried out in Buffer A (50 mM NaHEPES (pH 8.0), 1 mM EDTA, 1 mM dithiothreitol) that contained the concentrations of nucleotide and Mg2+ shown in the text. G, was ADP-ribosylated by the method of Katada et al. (17).

G proteins were reconstituted into phospholipid vesicles essentially according to the method of Sternweis (18). Purified G protein in 500 µl of Buffer A plus 0.02% Lubrol 12A9 was mixed with 100 µl of Buffer A plus 0.167% phospholipid and 0.84% cholate. The lipid was either dioleoyl-PC or a mixture of 0.05% dioleoyl-PC, 0.067% bovine brain PS, and 0.005% bovine brain PE. The suspension was held at

6491
RESULTS AND DISCUSSION

The effect of mastoparan on the activation of a G protein by GTPyS was tested using purified Gα, a G protein from bovine brain that is a substrate for ADP-ribosylation by pertussis toxin and that is known to promote IP3 production (6). As shown in Fig. 1, 100 μM mastoparan stimulated the rate of binding of GTPyS to Gα about 7-fold in the presence of 5 mM Mg2+, which is typical of similar experiments. The maximal amount of GTPyS binding was unaltered by the addition of mastoparan and was equal to the total amount of active Gα, present. The decrease in the amount of GTPyS bound at long times in the presence of 100 μM mastoparan is apparently due to the denaturation of Gα. Such a decrease was not noted in the presence of 10 μM mastoparan, which accelerated nucleotide binding by 2.5-fold.

The dissociation of GDP is potentially rate limiting in G protein activation (15). As shown in Fig. 1, mastoparan stimulated the dissociation of GDP from Gα, approximately 8-fold, to a rate that was adequate to account for the observed stimulation of GTPyS binding.

The concentration of mastoparan required to stimulate GTPyS binding to Gα was estimated in the experiment of Fig. 2, using 60-s time points to approximate initial rates of binding. The highest rates observed, kmax ~ 2 min−1, are 2-3-fold higher than those reported previously for initially GDP-ligated Gα (15, 16) and are comparable to receptor-stimulated rates that were observed for reconstituted Gα (19). In the experiment shown, 3 μM mastoparan increased the rate of binding 2-fold in the mixed phospholipid vesicle system. Significant responses to this concentration are routinely observed with reconstituted Gα, but higher concentrations are required in Lubrol solution. Because the initial rate of GTPyS binding did not saturate with increasing mastoparan concentrations, it is not possible to estimate the affinity of Gα for mastoparan from these data (or from GTPase data; see below).

Furthermore, the rates of mastoparan-stimulated guanine nucleotide binding and release by G proteins are complex functions of the concentrations of Mg2+ (Fig. 3), mastoparan, and the common G protein βγ subunits. The use of more potent analogs of mastoparan should allow more quantitative measurements of Gα-mastoparan association.

Because G protein stimulation by mastoparan appears qualitatively similar to stimulation by receptors, we examined whether stimulation by mastoparan was sensitive to the ADP-ribosylation of the G protein α subunit by pertussis toxin. ADP-ribosylation inhibits receptor-stimulated guanine nucleotide exchange reactions but generally does not alter basal rates dramatically (8, 9, 20). As shown in Table I, pertussis toxin-catalyzed ADP-ribosylation of Gα inhibited mastoparan-stimulated binding by 50–60% but had a negligible effect on the basal rate of binding. Such partial inhibition is in part due to incomplete ADP-ribosylation, which was approximately 50% relative to GTPyS binding, but it may also reflect an intrinsically limited sensitivity of mastoparan stimulation to ADP-ribosylation.

3T. Higashijima, unpublished observations. See Ref. 5 for a review of the interacting effects of Gβγ, and Mg2+ on Gαs.
The effects of pertussis toxin-catalyzed ADP-ribosylation on GTPyS binding to reconstituted G0

G0 was ADP-ribosylated (ADP-rib) using pertussis toxin and 1 mM NAD. Control samples of G0 were treated identically with toxin except that NAD was omitted. G0 was then reconstituted into PC or PC/PE/PS vesicles. GTPyS binding was measured as described in the legend to Fig. 1 by incubation for 1 min at 30°C with (+MP) or without (CON) 100 μM mastoparan. The increment in binding caused by mastoparan is also shown (Δ). The data shown are the average of triplicate assays. The total GTPyS binding activity in each assay was 1.49 and 1.45 pmol for the PC and PC/PS/PE vesicles, respectively.

<table>
<thead>
<tr>
<th>lipid</th>
<th>ADP-rib</th>
<th>bound [35S]GTPyS pmol/assay</th>
<th>CON</th>
<th>+MP</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>−</td>
<td>0.15</td>
<td>1.13</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>+</td>
<td>0.13</td>
<td>0.62</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>PE/PC/PS</td>
<td>−</td>
<td>0.068</td>
<td>1.10</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>PE/PC/PS</td>
<td>+</td>
<td>0.072</td>
<td>0.49</td>
<td>0.41</td>
<td></td>
</tr>
</tbody>
</table>

The α subunits of different G proteins are structurally homologous, and G protein-coupled receptors are not absolutely selective for the α subunits with which they interact (6, 20, for example). Similarly, mastoparan promoted nucleotide exchange by Gα, Gα, and transducin in addition to Gα (Table II). In several experiments, mastoparan stimulated steady-state GTP hydrolysis (Table II) and the rate of GTPyS binding (not shown) by rabbit hepatic Gα by 8-10-fold, although at slightly higher concentrations than had been observed with Gα. Mastoparan was less active in stimulating Gα and transducin. Mastoparan stimulated transducin only 2-fold and only at high concentrations. Although the GTPase data in Table II suggest that mastoparan stimulated Gα nearly 4-fold, this probably represents a significant contribution from contaminating Gα in the preparation. When mastoparan-stimulated activation of Gα by GTPyS was assayed according to the ability of activated Gα to stimulate adenylyl cyclase (21), only 2-fold stimulations of the activation rate were observed. However, mastoparan can stimulate adenylyl cyclase activity in plasma membranes of S49 lymphoma cells (not shown), suggesting that its activity on Gα in native systems may be significant. The apparent selectivity of mastoparan for Gα and Gβ over transducin and Gα may reflect true differences in affinity or may be caused by differences among G proteins in the conditions that are optimal for a response. Preliminary data suggest that analogs of mastoparan may display relative selectivities among Gα subunits that differ from native mastoparan.

G proteins are much more sensitive to mastoparan when reconstituted into phospholipid vesicles than when in detergent solution. The potency and/or efficacy of mastoparan was also increased at least 3-fold, and sometimes more than 10-fold, by reconstitution (Fig. 2). This situation is similar to that observed with receptor-mediated stimulation of nucleotide binding, which occurs essentially only when both proteins are membrane bound. Such potentiation of the effect of mastoparan by reconstitution is also the case for Gα and Gβ (data not shown).

Receptor-catalyzed nucleotide exchange by G proteins occurs at micromolar concentrations of Mg2+, while 10–100 mM Mg2+ is optimal for exchange in the absence of receptor and agonist (see Ref. 5). As shown in Fig. 3, mastoparan was fully active at the low concentrations of Mg2+ that are characteristic of receptor-mediated events. The effect of mastoparan was maximal at 1–10 μM Mg2+, where GTPase activity was stimulated about 20-fold.

Because the effect of mastoparan on G protein activation bears several similarities to the action of hormone receptors, it is tempting to suggest that mastoparan may interact with G proteins at a conserved receptor-binding domain in a manner structurally as well as functionally similar to that of hormone receptors. Recently, the primary structures of several homologous G protein-coupled receptors have been determined (Ref. 22 for review). Each receptor apparently spans the bilayer seven times, exposing to the cytoplasm two small, positively charged helices, a larger hydrophilic loop, and the carboxyl-terminal domain. Recent studies suggest that neither the carboxyl-terminal region nor most of the large intracellular loop is required for interaction with G proteins (23, 24). Homology arguments suggest the importance of the two small, basic loops in regulating G protein function (22).

When mastoparan binds to phospholipid membranes, it forms a highly structured α-helix that exposes its four positive charges to the aqueous medium (25, 26). This structural motif is reminiscent of the two basic intracellular loops of the receptors. An ordered array of positive charges close to the surface of the bilayer may thus be a salient characteristic of both G protein-coupled receptors and of mastoparan. The use of modified mastoparans with enhanced potency and improved selectivity for specific G proteins should help resolve whether this is in fact the G protein-binding site.

It is likely, but not certain, that mastoparan stimulates the release of histamine and other biological regulators primarily via its effect on G proteins. The sensitivity of mastoparan-induced histamine release to the effect of pertussis toxin (7) argues that a G protein is directly involved. The potency of mastoparan in stimulating the GTPase activity of Gα is similar to its potency as a secretagogue (7), and structure-activity relationships for histamine release and for G protein activation by several mastoparan analogs are consistent. Conversely, the affinities of mastoparan analogs for either calmodulin or lipid bilayers do not correlate with secretagogue activity. Because mastoparan is an amphipathic cation, it may cross the plasma membrane in response to the membrane potential and thus interact directly with G proteins on the cytoplasmic face. Such electrophoretic transfer across a lipid bilayer has been demonstrated for melittin, another basic, amphipathic peptide (27). The use of mastoparan and its analogs to study secretory processes and other G protein-mediated events in cells may thus be feasible and instructive.

The effect of mastoparan on G proteins points out that G protein-mediated signaling pathways are conspicuous targets for diverse biological toxins. Pertussis toxin, cholera toxin, and a botulinum toxin all catalyze the ADP-riboseylation of diverse G protein α subunits (5, 28). Invasive adenylyl cyclases from Bordetella pertussis and Bacillus anthracis (29, 30) and phospholipases in snake venom carry out what are nor-
Mastoparan Stimulation of G Proteins

mally G protein-regulated functions. The ability of mastoparan to mimic G protein-coupled receptors both structurally and functionally represents a novel toxic attack on transmembrane signaling.

Acknowledgments—We are grateful to Dr. Tatsuo Miyazawa for discussion and support during the initiation of this study. We thank Dr. Toshiaki Katada for helpful discussions and Jimmy Woodson for excellent technical assistance.

REFERENCES