BIOPHYSICS AND BIOCHEMISTRY

Molecular Mechanisms for the Effect of Mastoparan on G Proteins in Tissues of Vertebrates and Invertebrates

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 3, pp. 273-277, March, 2006
Original article submitted May 6, 2005

The peptide toxin mastoparan increased GTP-binding activity of heterotrimeric G proteins in tissues of vertebrate and invertebrate animals, the effect of mastoparan in mussel tissues being less pronounced. The stimulatory effect of mastoparan on GTP binding was not observed after treatment of membranes with pertussis toxin that selectively modulates function of G_i proteins. Activity of mastoparan decreased in the presence of C-terminal peptide 346-355 from the G_i protein α_{i2}-subunit. Mastoparan dose-dependently decreased the stimulatory effect of hormones on GTP binding in tissues of rats and mussels. The influence of these hormones on the cell is realized via G_i proteins. However, mastoparan did not modulate the effect of G_s protein-activating hormones.

Key Words: *mastoparan; myocardium; somatostatin; G protein; GTP binding*

Studying the molecular mechanisms for the effect of hormones on cellular effector systems is a priority problem of modern molecular endocrinology. The interaction of the hormone-activated receptor with heterotrimeric G protein followed by stimulation of its GTP-binding and GTPase activities is an important stage in intracellular hormone signal transduction. This process involves proximal membrane segments of the receptor cytoplasmic loop and C-terminal regions of G protein α-subunits. Some nonhormonal substances also can stimulate G proteins by a receptor-independent mechanism (*e.g.*, polycationic helical peptides) [1-3,7,9,15]. These substances mimic positively charged regions of receptor cytoplasmic loops that interact with the negatively charged receptor-binding sites in G proteins. Polycations not only activate G proteins, but also impair functional coupling between receptor-activated hormone and G protein. They block hormone signal transduction to the effector systems. Mastoparans, peptide toxins isolated from insect venom, belong to these nonhormonal regulators [7]. The toxic effect of these peptides on mammalian tissues is associated with stimulation of G proteins. Mastoparan can be used in studies of the molecular mechanisms for functional coupling between receptors and G proteins. Moreover, these substances hold promise in developing new-generation drugs modulating or regulating activity of hormonal signal systems.

The effects of mastoparans on functional activity of hormonal signal systems (*e.g.*, G proteins) in invertebrate animals are little studied. This influence should be evaluated not only for deciphering of the molecular mechanisms for functional coupling between G protein and receptor, but also for identification of molecular determinants responsible for this coupling. The molecular mechanisms underlying the effects of mastoparans and synthetic

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polycationic peptides remain unknown. Some data exist on nonpeptide polycations. Compounds C48/80 and N-alkyl-substituted lysine derivatives directly interact with inhibitory G proteins (less significantly than mastoparan) [6,8].

Here we compared the molecular mechanisms underlying the effect of mastoparan from wasp venom (*Polistes jadwagae*) on basal and hormone-stimulated (biogenic amines and somatostatin) GTP-binding activity of G proteins in tissues of phylogenetically different animals (mussels and rats). We compared the influence of mastoparan and synthetic polycationic peptides [1-3]. The main goal of this study was to identify G proteins that serve as the target for mastoparan and synthetic peptides.

MATERIALS AND METHODS

The plasma membrane fraction of rat myocardium and brain striatum (*Rattus norvegicus*) and smooth muscles of bivalve mussels (*Anodonta cygnea*) were isolated as described elsewhere [5,12]. Each fraction was obtained from 5-7 rats and 15-20 mussels.

Experiments were performed with *Polistes jadwagae* mastoparan, pertussis toxin (PT), somatostatin, serotonin, isoproterenol, bromocryptine (Sigma), and other reagents (Sigma and Reanal). The peptides corresponding to C-terminal regions 385-394 (α_s-subunit) and 346-355 (α_{i2}-subunit) of human G proteins and polycationic peptides C-εAhx-WKK(C_{10})-KKK(C_{10})-KKKK(C_{10})-YKK(C_{10})-KK (peptide I) and (GRGDSGRKKRRQRRRPPQ)$_2$-K-εAhx-C(Acm) (peptide II) were synthesized as described previously [1-4]. eAhx, C_{10}, and Acm are the residue of ε-aminohexanoic acid, residue of capric acid, and acetamide group, respectively. GTP-binding activity of G proteins was estimated with β,γ-imido[8-^{3}H]-guanosine-5'-triphosphate ammonium salt ([8-^{3}H]-GppNHp, 5 Ci/mmol, Amersham) using type HA nitrocellulose filters (0.45 μ, Millipore).

Specific GTP-binding activity of G proteins was calculated as the difference between binding of labeled [8-^{3}H]Gpp[NH]p in the absence or presence of 10 mM GTP [4].

The method for ADP ribosylation of plasma membrane fractions with PT was described elsewhere [12]. The reaction was conducted at 37 (rats) or 30°C (mussels) for 45 min. PT in a concentration of 10 mg/ml was added to the incubation medium (400 ml). The incubation medium contained 50 mM Tris-HCl (pH 7.8), 2 mM $MgCl_2$, 1 mM EDTA, 10 mM dithiothreitol (DTT), 0.1 mM NAD, 1 mM NADP, 0.1 mM GTP, 1 mM ATP, and 10 mM thymidine. Membrane protein concentration was 0.95-1.00 mg/ml. PT was preactivated with DTT and ATP at 37 (rats) or 30°C (mussels) for 15 min. The reaction of ADP ribosylation was stopped by adding cold HCl-buffer (5 ml, 50 mM, pH 7.5). The samples were centrifuged at 100,000*g* for 30 min. The precipitate of ADP-ribosylated membranes was resuspended in the same buffer and used to measure GTP-binding activity. Control samples were treated in the absence of PT.

The results were analyzed by means of ANOVA software. Each experiment was performed in 3 repetitions. The data are expressed as means and standard errors (several independent experiments). The differences were significant at $p<0.05$.

RESULTS

Mastoparan in concentrations of 1.0×10^{-5}-2.5×10^{-4} M increased GTP-binding activity of G proteins in the myocardium and brain striatum of rats, as well as in smooth muscles of *A. cygnea* mussels (Fig. 1). The effect of mastoparan in rat tissues was more pronounced than in mussels. Synthetic polycationic peptides I and II in a concentration of 10^{-5} M increased GTP-binding activity in the myocardium (by 118 and 59%, respectively), striatum (by 139 and 74%, respectively), and smooth muscles (by 88 and 43%, respectively). These data show that natural and synthetic polycationic peptides have a direct effect on G proteins and increase GTP-binding activity.

PT selectively modified the cysteine residue localized near the C-terminal fragment of $G_{i/o}$ protein

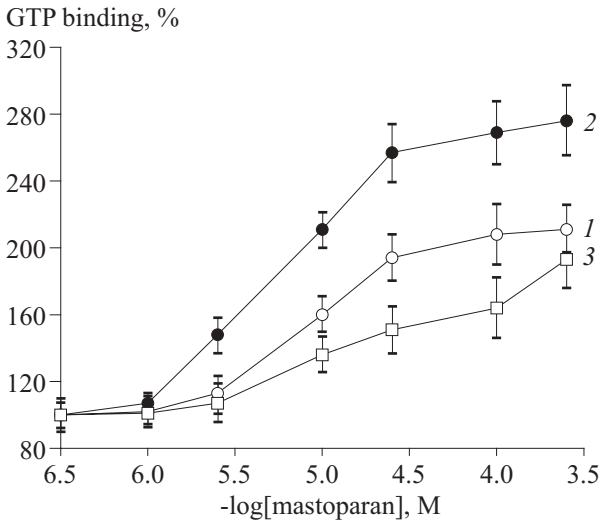

Fig. 1. Stimulatory effect of mastoparan on GTP binding in plasma membranes from rat and mussel tissues. Rat myocardium (*1*), rat brain striatum (*2*), and mussel smooth muscles (*3*). Basal level of GTP binding (rat myocardium, 2.37±0.14 pmol [8-^{3}H]-GppNHp per mg protein; rat brain striatum, 6.93±0.35 pmol [8-^{3}H]-GppNHp per mg protein; mussel smooth muscles, 2.95±0.24 pmol [8-^{3}H]-GppNHp per mg protein) is taken as 100%.

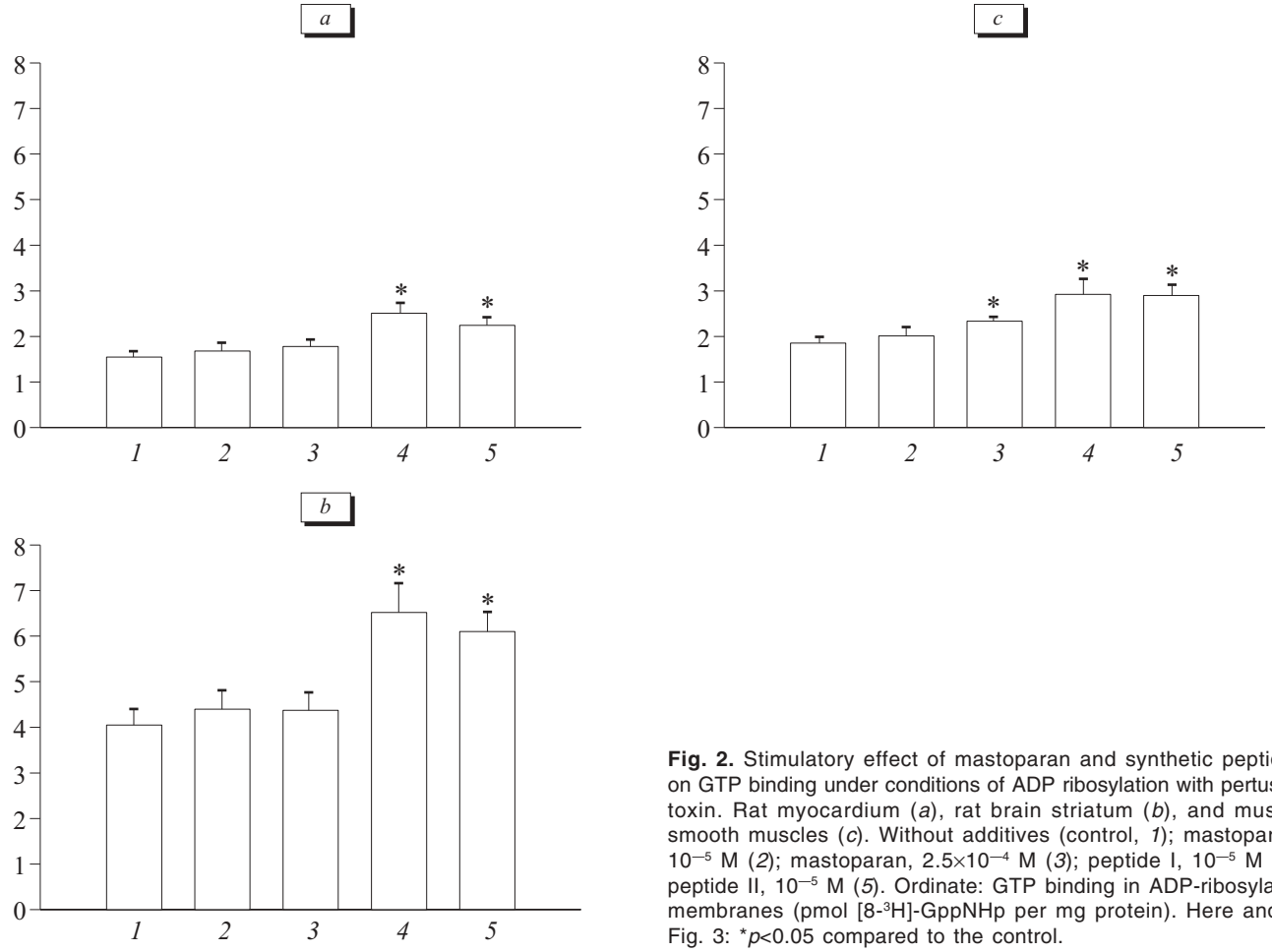

Fig. 2. Stimulatory effect of mastoparan and synthetic peptides on GTP binding under conditions of ADP ribosylation with pertussis toxin. Rat myocardium (*a*), rat brain striatum (*b*), and mussel smooth muscles (*c*). Without additives (control, *1*); mastoparan, 10^{-5} M (*2*); mastoparan, 2.5×10^{-4} M (*3*); peptide I, 10^{-5} M (*4*); peptide II, 10^{-5} M (*5*). Ordinate: GTP binding in ADP-ribosylated membranes (pmol [8-^{3}H]-GppNHp per mg protein). Here and in Fig. 3: **p*<0.05 compared to the control.

α-subunits, which impairs their functional coupling to the activated receptor [13]. PT treatment of membranes abolished the stimulatory effect of mastoparan in a concentration of 10^{-5} M on GTP binding (Fig. 2). Mastoparan in higher concentrations (2.5×10^{-4} M) had a weak stimulatory effect on GTP binding in PT-treated membranes from mussel muscles. These data indicate that mastoparan selectively activates $G_{i/o}$ proteins in tissues of rats and mussels. It should be emphasized that mastoparan in relatively high concentrations slightly stimulates other types of G proteins in mussel muscles. The stimulatory effect of peptides I and II in PT-treated membranes decreased by 55% (Fig. 2), which attests to low selectivity of their binding with G proteins. The stimulatory effect of peptide I was more sensitive to PT treatment. Peptide I was 2-fold more potent than peptide II in producing the stimulatory effect in control membranes. However, in ADP-ribosylated membranes their effects little differed.

Elimination of the effect of mastoparan upon treatment with PT suggests that the C-terminal region of $G_{i/o}$ protein α-subunits is involved in its interaction with G protein. The stimulatory effect of mastoparan decreased in the presence of C-terminal peptide 346-355 of the G_i protein α_{i2}-subunit (Table 1), while peptide 385-394 of G_s protein α_s-subunit did not modulate the effect of mastoparan in rat tissues, but slightly decreased it in mussel muscles. C-terminal peptides selectively and competitively impair signal transduction via G proteins from which they are derived [4,10,14]. The stimulatory effect of peptides I and II on GTP binding was sensitive to the presence of both C-terminal peptides. The effect of peptide I in rat tissues decreased more significantly in the presence of peptide 346-355 of α_{i2}-subunit. However, the effect of peptide II in various tissues decreased similarly in the presence of both C-terminal peptides. These data indicate that the formation of a polycationic amphipathic helix by the peptides is not essential and sufficient for selective activation of certain G proteins (*e.g.*, G_i proteins). This process depends on the primary structure of peptides, which determines the distribution of positively charged groups on the helix surface.

Mastoparan in concentrations of 10^{-5} and 2.5×10^{-4} M dose-dependently suppressed the stimulating GTP-binding effect of hormones realized

via G_i proteins. We observed a decrease in the effects of somatostatin (myocardium), somatostatin and D_2 dopamine receptor agonist bromocryptine (brain striatum) and, to a lesser extent, of isoproterenol (mussel muscles, Fig. 3). G_i proteins are involved in the regulatory effect of these hormones on effector components of signal systems. The influence of hormones was completely (somatostatin) or partially suppressed (bromocryptine and isoproterenol) after treatment with PT. Hormonal activity decreased in the presence of peptide 346-355 from the α_{i2}-subunit. Previous studies showed that bromocryptine and isoproterenol decrease adenylate cyclase activity in rat brain striatum [4] and mussel smooth muscles [11], respectively.

Mastoparan even in a concentration of 2.5×10^{-4} M did not modulate the stimulatory effect of isoproterenol and serotonin on GTP binding realized via G_s proteins in rat myocardium and striatum, respectively (data not shown). Mastoparan decreased the stimulatory effect of serotonin in muscles of *A. cygnea* mussels (by 16%). Probably, mastoparan exhibits lower selectivity for G proteins

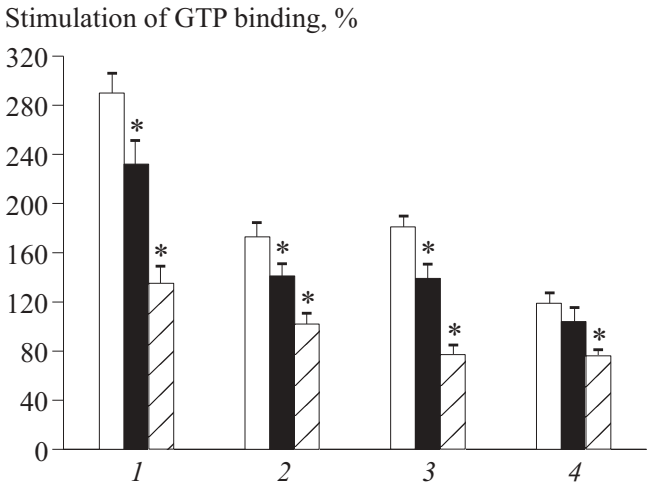

Fig. 3. Effect of mastoparan on the stimulatory influence of hormones on GTP binding realized via G_i proteins in rat myocardium (*1*), rat brain striatum (*2*, *3*), and mussel smooth muscles (*4*). Somatostatin, 10^{-7} M (*1*, *2*); bromocryptine, 10^{-5} M (*3*); isoproterenol, 10^{-5} M (*4*). Light bars, hormone (control); dark bars, hormone and mastoparan (10^{-5} M); shaded bars, hormone and mastoparan (2.5×10^{-4} M). Ordinate: stimulatory influence of hormone on GTP binding (%).

TABLE 1. Effect of C-terminal Peptides 346-355 of G Protein α_{i2}-Subunit and 385-394 of G Protein α_s-Subunit on the Stimulatory Influence of Mastoparan and Synthetic Peptides on GTP Binding (*M*±*m*, *n*=6)

Treatment	GTP binding, pmol [8-^{3}H]-GppNHp per mg protein		
	without peptides	α_{i2} peptide 346-355, 10^{-4} M	α_s peptide 385-394, 10^{-4} M
Rat myocardium			
Without additives	2.65±0.18	2.50±0.25	2.47±0.17
Mastoparan, 10^{-5} M	4.43±0.28 (67%)*	3.42±0.26 [37%]*	4.22±0.37 [71%]*
Mastoparan, 2.5×10^{-4} M	5.51±0.46 (108%)*	4.18±0.40 [67%]*	5.20±0.35 [111%]*
Peptide I, 10^{-5} M	5.54±0.52 (109%)*	4.10±0.31 [64%]*	4.72±0.44 [91%]*
Peptide II, 10^{-5} M	4.28±0.51 (62%)*	3.75±0.28 [50%]*	3.80±0.24 [54%]*
Rat brain striatum			
Without additives	7.25±0.58	6.98±0.35	7.20±0.52
Mastoparan, 10^{-5} M	14.57±1.05 (101%)*	11.02±0.90 [58%]*	14.19±1.20 [97%]*
Mastoparan, 2.5×10^{-4} M	18.42±1.76 (154%)*	12.84±1.09 [84%]*	17.69±1.85 [146%]*
Peptide I, 10^{-5} M	16.31±1.26 (125%)*	12.13±1.00 [74%]*	13.60±0.95 [89%]*
Peptide II, 10^{-5} M	12.43±1.33 (71%)*	10.65±1.02 [53%]*	11.12±0.91 [54%]*
Mussel smooth muscles			
Without additives	3.14±0.30	3.02±0.19	3.17±0.18
Mastoparan, 10^{-5} M	4.41±0.34 (40%)*	3.80±0.22 [26%]*	4.21±0.32 [33%]*
Mastoparan, 2.5×10^{-4} M	6.05±0.35 (93%)*	4.85±0.31 [61%]*	5.48±0.49 [73%]*
Peptide I, 10^{-5} M	5.78±0.27 (84%)*	5.12±0.34 [70%]*	5.09±0.23 [61%]*
Peptide II, 10^{-5} M	4.35±0.31 (39%)*	3.88±0.21 [28%]*	3.92±0.40 [24%]*

Note. Stimulatory effect of mastoparan and synthetic peptides on GTP binding is shown in parentheses (compared to the basal level). Stimulatory effect of test substances relative to GTP binding in the presence of C-terminal peptides is shown in square brackets. *$p<0.05$ compared to the parameter observed without additives.

in mussels (Fig. 2, Table 1). It can be hypothesized that mastoparan dose-dependently decreases the stimulatory effect of hormones on GTB binding. It should be emphasized that the regulatory influence of these hormones is realized via G_i proteins. However, mastoparan does not modulate the effect of G_s protein-activating hormones.

As differentiated from synthetic polycationic peptides producing the nonselective effect, mastoparan selectively interacts with G_i proteins in tissues of rats and mussels. These data indicate that the effects of mastoparan on G proteins in tissues of vertebrate and invertebrate animals are realized via similar molecular mechanisms. Mastoparan holds much promise for the studies of signal mechanisms underlying the influence of hormones (peptides, biogenic amines, *etc*.) on hormone signaling systems in tissues of phylogenetically different animals.

This work was supported by the Russian Foundation for Basic Research (grant No. 03-04-49114).

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