British Journal of Pharmacology (1997) 122, 31–36

G protein-independent stimulation of human myocardial phospholipase C by mastoparan

[1]Petra Schnabel, Heidi Gäs, Theo Nohr & Michael Böhm

Klinik III für Innere Medizin, Universität zu Köln, Germany

1 Phosphoinositide-specific phospholipase C (PLC) is involved in the regulation of many cellular functions. In the myocardium, PLC-generated second messengers play a role in the regulation of contractile function and in the pathophysiology of myocardial hypertrophy.

2 In the present study, the effect of mastoparan, a tetradecapeptide which is capable of activating heterotrimeric G proteins by mimicking the action of an activated receptor, on membrane-bound human myocardial PLC, was investigated in a cell-free assay with exogenous phospholipids as a substrate.

3 Mastoparan stimulated human myocardial PLC approximately two fold with a half-maximal effect at approximately 2 μM and a maximal effect at 10 μM. The peptide did not alter the dependence of PLC on free calcium ions. In order to exclude non-specific effects of mastoparan due to its amphiphilic properties, different mastoparan derivatives were used as positive and negative controls. Mas17, an inactive mastoparan analogue with phsyical properties very similar to mastoparan, did not induce substantial PLC stimulation in human myocardial membranes. In contrast, Mas7, the most active mastoparan derivative known, caused a more pronounced PLC activation compared with the mother compound indicating that the effect was sequence-specific. Human myocardial PLC stimulation was pertussis toxin-insensitive and could not be abolished by addition of excess α-subunits from purified retinal transducin or by excess GDP or GDPβS. In order to investigate whether mastoparan stimulated PLC via pertussis toxin-insensitive α_q, a deletion mutant of PLCβ_2 deficient of the site of interaction with α_q-subunits was expressed in COS-1 cells. Both wild-type and mutant PLCβ_2 were similarly sensitive to stimulation by mastoparan.

4 It is concluded that mastoparan stimulates human myocardial PLC by a mechanism distinct from heterotrimeric G proteins.

Keywords: Mastoparan; phospholipase C; G proteins; signal transduction; human myocardium

Introduction

Phosphoinositide-specific phospholipase C (PLC) generates two intracellular second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($InsP_3$) by hydrolysis of phosphatidylinositol 1,4,5-bisphosphate ($PtdInsP_2$). DAG stimulates protein kinase C (PKC) and thus initiates a complex phosphorylation cascade (Nishizuka, 1992 for review), while $InsP_3$ releases calcium from intracellular stores (Berridge, 1993 for review). Many extracellular signalling molecules like hormones, neurotransmitters and growth factors induce their biological response via activation of PLC enzymes. In the mammalian heart, PLC takes part in the regulation of contractile force by influencing intracellular calcium homeostasis through $InsP_3$. The most widely studied receptor coupling a stimulus (noradrenaline) to a positive inotropic response is the α-adrenoceptor (Terzic *et al*., 1993 for review). Moreover, signal transduction of α-adrenoceptor-mediated stimuli by PLC appears to be an important mechanism in the pathogenesis of myocardial hypertrophy (Simpson, 1983). A number of additional hypertrophic stimuli such as angiotensin II and endothelin are also coupled to PLC.

To date, the cDNAs of ten PLC isozymes have been cloned from mammalian tissues (Rhee & Choi, 1992 for review). All of them contain two highly homologous regions of 170 and 250 residues, respectively, which are designated X and Y and believed to correspond to the catalytic domain. According to their similarity outside the X and Y regions PLC isozymes are divided into three families designated PLCβ, PLCγ and PLCδ. The PLCβ family comprises four members which are regulated by heterotrimeric G proteins. PLCβ_4 is the most distantly related isozyme and is expressed mainly in the retina and in neuronal tissues (Kim *et al*., 1993; Lee *et al*., 1993; 1995). In contrast, PLCβ_{1-3} are more closely related with respect to their amino acid sequence and their sensitivity to stimulation by both G protein α- and $\beta\gamma$-subunits amino acid sequence and their sensitivity to stimulation by both G protein α- and $\beta\gamma$-subunits (Camps *et al*., 1992a, b; Katz *et al*., 1992; Park *et al*., 1993; Smrcka & Sternweis, 1993). In human myocardium, PLC activity has recently been shown to be stimulated by G protein α- and $\beta\gamma$-subunits (Schnabel *et al*., 1996).

Mastoparan, a tetradecapeptide isolated from wasp venom, activates G proteins by catalyzing guanosine 5′-diphosphate/guanosine 5′-triphosphate (GDP/GTP) exchange. Thus the compound mimics the action of activated G protein-coupled receptors (Higashijima *et al*., 1988). Mastoparan is a widely used tool to study G protein-mediated signal transduction events (Ross & Higashijima, 1994 for review). The peptide has been shown to stimulate PLC in certain cellular systems like rat mast cells (Mousli *et al*., 1989), rat hepatocytes (Tohkin *et al*., 1990; Garcia-Sainz *et al*., 1991), human HL-60 leukaemia cells (Gusovski *et al*., 1991; Norgauer *et al*., 1992), and rat PC-12 phaeochromocytoma cells (Choi *et al*., 1992). In contrast, inhibition of PLC by mastoparan has been demonstrated in SH-SY5Y human neuroblastoma cells (Wojcikiewicz & Nahorski, 1989) and in human astrocytoma cells (Nakahata *et al*., 1990). The present study was initiated to investigate whether mastoparan induces inhibition or stimulation of PLC in human myocardium, and, in addition, to elucidate the mechanism by which the peptide exerts its effect. In our experiments, a stimulating effect of mastoparan on human myocardial PLC was established.

[1]Author for correspondence at: Klinik III für Innere Medizen, Universität zu Köln, Joseph-Stelzmann-Str. 9, 50924 Köln, Germany.

Surprisingly, this stimulation appeared to be independent of G protein α- and $\beta\gamma$-subunits.

Methods

Myocardial tissue

Human myocardial tissue was obtained during cardiac transplantation of patients with terminal heart failure due to dilated cardiomyopathy. Cardiac surgery was performed on cardiopulmonary bypass. Cardioplegia was induced by a modified Bretschneider solution (mM: NaCl 15, KCl 10, $MgCl_2$ 4, histidine HCl 180, tryptophan 2, mannitol 30 and potassium dihydrogen oxoglutarate 1). Explanted hearts were transported from the operation room to the laboratory within five minutes and snap-frozen in liquid nitrogen. Medical therapy of the patients consisted of digitalis, diuretics, nitrates and angiotensin converting enzyme inhibitors. None of the patients had received catecholamines or β-blockers.

Membrane preparation

Left ventricular myocardial tissue was thawed on ice, minced with scissors and homogenized in 10 volumes TED buffer (50 mM Tris-HCl pH 7.5; 1 mM EDTA; 1 mM dithiothreitol) by a polytron for 30 s. This homogenate was centrifuged at $480 \times g$ in a Beckman JA-20 rotor at 4°C for 15 min. The supernatant was incubated with TED buffer containing a final concentration of 500 mM KCl on ice for 30 min. Subsequently, the homogenate was centrifuged at $48{,}000 \times g$ in a Beckman JA-20 rotor at 4°C for 30 min. The pellet was resuspended in the same volume of TED buffer without KCl and recentrifuged at $48{,}000 \times g$ at 4°C for 30 min. This washing step was repeated twice. The final pellet was resuspended in 1 volume TED buffer and snap-frozen in liquid nitrogen.

Purification of α_t and $\beta\gamma_t$

The purification of α_t and $\beta\gamma_t$ from bovine rod outer segments has been described previously (Camps *et al*., 1992b). Briefly, heterotrimeric tranducin was eluted from rod outer segment membranes with hypotonic buffer containing 100 μM GTP and separated into α and $\beta\gamma$ subunits by chromatography on Blue Sepharose CL-6B (Pharmacia-LKB). The purity of the proteins was at least 95%, as judged by analysis of silver-stained sodium dodecyl sulphate (SDS)/polyacrylamide gels.

Culture and transfection of COS-1 cells

COS-1 cells were grown in Dulbecco's modified Eagle Medium supplemented with 10% foetal calf serum and transfected with plasmid DNA by lipofection with Transfectam (Promega) as described previously (Camps *et al*., 1992a; Schnabel *et al*., 1993). Forty eight hours after transfection, cells were harvested and cholate extracts were produced as described previously (Camps *et al*., 1992a; Schnabel *et al*., 1993).

Phospholipase C assay

Inositol phosphate formation was assayed for 30 min at 25°C by use of exogenous phospholipid vesicles containing [^{3}H]-phosphatidylinositol 4,5-bisphosphate ($PtdInsP_2$) as substrate (Camps *et al*., 1992b; Schnabel *et al*., 1993; 1996). The reaction mixture (70 μl) contained 280 μM phosphatidylethanolamine, 28 μM [^{3}H]-$PtdInsP_2$ (5 Ci mol^{-1}), 50 mM Tris/maleate pH 7.0, 10 mM LiCl, 10 mM 2,3-diphosphoglycerate, 3 mM EGTA, 5 mM $MgCl_2$, 1 mM ATP and 0.9 mM sodium deoxycholate unless indicated differently in the figure legends. The free Ca^{2+} concentrations are given in the figure legends. The reaction was terminated by addition of 350 μl chloroform/methanol/concentrated HCl (500/500/3 by volume) and vortexing. Subsequently, 100 μl 1 M HCl containing 5 mM EGTA were added. Phase separation was accelerated by centrifugation at $12{,}000 \times g$ for 1 min in an Eppendorf microfuge; 200 μl of the aqueous phase were counted in a Beckman scintillation counter in Zinsser Quicksafe scintillation fluid.

Miscellaneous

SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting were performed as described previously (Camps *et al*., 1992a; Schnabel *et al*., 1993) with rabbit antisera raised against the carboxy terminal pentadecapeptide of PLCβ_2.

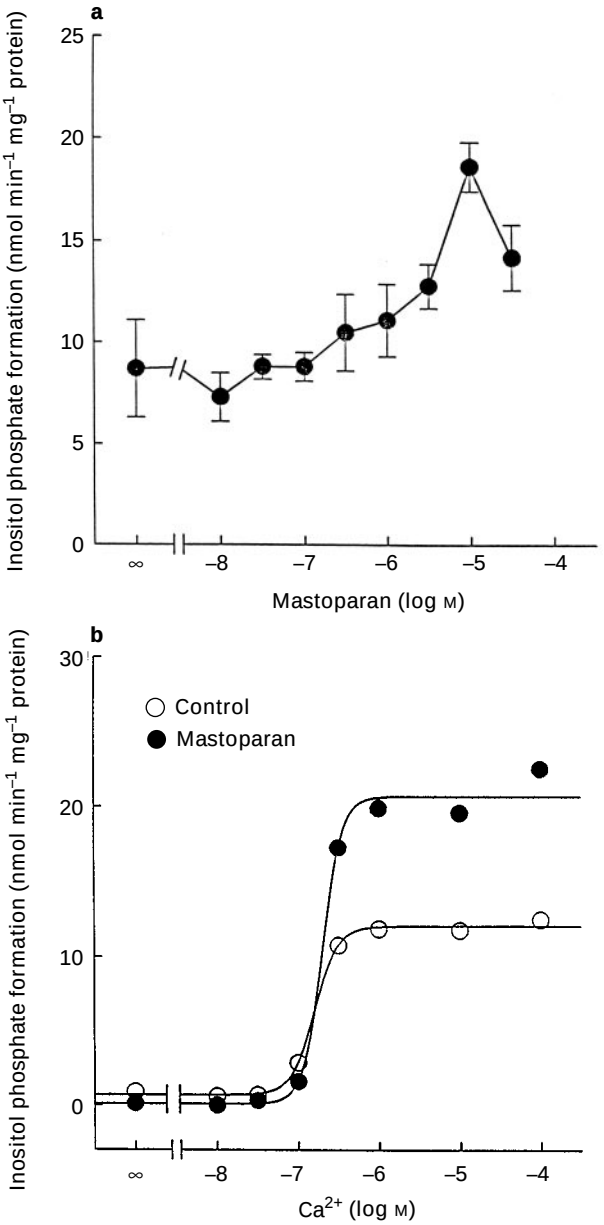

Figure 1 (a) Concentration-dependence of effect of mastoparan on inositol phosphate formation by human myocardial membranes. Membrane preparations from human myocardium (2 μg protein/tube) were incubated with phospholipid vesicles containing $PtdInsP_2$ for 30 min at 25°C in the presence of 100 μM Ca^{2+}. The values correspond to means of triplicate determinations; vertical lines show s.d. (b) Concentration-dependence of effect of free Ca^{2+} on inositol phosphate formation by human myocardial membranes. Membrane preparations from human myocardium (2 μg protein/tube) were incubated with phospholipid vesicles containing $PtdInsP_2$ for 30 min at 25°C in the absence and presence of 10 μM mastoparan at the Ca^{2+} concentrations indicated on the abscissa scale. The values correspond to the means of duplicate determinations. In (a) and (b): the experiments shown are representative of three experiments performed.

Proteins were visualized by means of the ECL Western blotting detection system. Protein concentrations were determined according to Lowry *et al*. (1951) with bovine serum albumin as standard.

Materials

Mastoparan was purchased from Bachem (Heidelberg, F.R.G), Mas7 from Biomol (Hamburg, F.R.G.) and Mas 17 from Peninsula (Heidelberg, F.R.G.). All other materials were obtained from standard vendors or from sources previously described (Camps *et al*., 1992a; Schnabel *et al*., 1993).

Results

Phospholipase C activity present in human myocardium has previously been shown to be regulated by heterotrimeric G proteins (Schnabel *et al*., 1996). In order to characterize the stimulation of myocardial PLC by G protein subunits in more detail, the effects of mastoparan, a tetradecapeptide from wasp venom, was investigated. Mastoparan is known to activate heterotrimeric G proteins, particularly G_i and G_o, by mimicking the action of an activated receptor (Higashijima *et al*., 1988; 1990). Membrane preparations from human left ventricular myocardium were incubated with exogenous phospholipid vesicles containing [^{3}H]-PtdInsP_2 as substrate and with increasing concentrations of mastoparan (Figure 1a). There was a concentration-dependent stimulating effect on PLC with the maximal effect observed with 10 μM mastoparan. At 10 μM mastoparan, stimulation of myocardial PLC was about two fold. The stimulation was statistically highly significant (21.9 ± 2.3 nmol min^{-1} mg^{-1} protein at 10 μM mastoparan vs 11.7 ± 1.6 nmol min^{-1} mg^{-1} protein in the absence of mastoparan; $n=9$; $P=0.0022$ by two-tailed Student's t test for unpaired observations). The half-maximal effect was reached at about 2 μM of the peptide.

In order to investigate whether mastoparan altered the affinity of human myocardial PLC for calcium, the concentration-dependence of human myocardial PLC on free calcium ions was assessed in the absence and presence of 10 μM mastoparan. Under both conditions, PLC activity was strictly dependent on and substantially stimulated by calcium (Figure 1b). Maximal PLC activity was observed at 1 μM calcium with half-maximal activity at approximately 200 nM calcium. These results indicate that mastoparan does not influence the affinity of human myocardial PLC for calcium.

Mastoparan is a tetradecapeptide with amphiphilic properties. In order to distinguish whether the stimulant effect of mastoparan was due to its amphiphilicity or a specific effect of the peptide, a positive and a negative control were included in the experiments. Mas7 is the most active mastoparan analogue known (Ross & Higashijima, 1994 for review), while Mas17 is inactive. Both have amphiphilic properties similar to the original peptide. Human myocardial membranes and the peptides (10 μM each) were incubated with the phospholipid substrate under the same conditions (Table 1). Mas7 stimulated human myocardial PLC more noticeably than mastoparan. In contrast, Mas17 was unable to induce a marked stimulation of PLC. These results indicate that mastoparan exerts a sequence-specific stimulant effect on myocardial PLC.

The stimulant of PLC by mastoparan was not abolished by pertussis toxin treatment indicating that the mastoparan effect is not mediated by pertussis toxin-sensitive G proteins (Table 2). As pertussis toxin-sensitive PLC activation observed in a variety of cell lines such as HL-60 cells was suggested to be due

Table 1 Stimulation of PLC activity from human myocardial membranes by mastoparan and its derivatives

	Inositol phosphate formation (nmol min^{-1} mg^{-1} protein)
Control	10.0 ± 0.5
Mas	29.8 ± 1.0
Mas7	35.0 ± 0.6
Mas17	14.3 ± 1.5

Preparations from human myocardial membranes (1 μg protein/tube) were incubated with phospholipid vesicles containing PtdInsP_2 for 30 min at 25°C at 1 μM Ca^{2+} under control conditions, in the presence of mastoparan (Mas; 10 μM), the active analogue Mas7 (10 μM) or the inactive analogue Mas17 (10 μM). The values correspond to means $\pm$ s.d. of triplicate determinations. The experiment shown is representative of three experiments performed.

Table 2 Effect of pertussis toxin on stimulation of human myocardial PLC by mastoparan

	Inositol phosphate formation (nmol min^{-1} mg^{-1} protein)	
	Control	*Mastoparan*
Control	12.7 ± 0.5	20.9 ± 1.3
Pertussin toxin	16.5 ± 0.3	27.1 ± 1.1

Human myocardial membranes (1 μg protein/tube) were treated with preactivated pertussis toxin (1 μg/tube) or control buffer in the presence of 50 mM, 2 mM ATP, 1 mM GTP and 3 mM NAD for 1 h at 37°C. Subsequently, the membranes were incubated with phospholipid vesicles containing PtdInsP_2 for 30 min at 25°C at 1 μM Ca^{2+} in the absence or presence of mastoparan (10 μM). The experiment shown is representative of three experiments performed.

Table 3 Effect of α_t on stimulation of human myocardial PLC by mastoparan

	Inositol phosphate formation (nmol min^{-1} mg^{-1} protein)	
	Control	*Mastoparan*
Control	11.5 ± 0.6	23.4 ± 0.6
α_t	12.0 ± 0.9	24.9 ± 1.3

Preparations from human myocardial membranes (1 μg protein/tube) were incubated with phospholipid vesicles containing PtdInsP_2 for 30 min at 25°C at 1 μM calcium under control conditions, in the presence of α_t 4 μM) alone, in the presence of mastoparan (10 μM) and in the presence of both α_t and mastoparan. The values correspond to means $\pm$ s.d. of triplicate determinations. The experiment shown is representative of three experiments performed.

Table 4 Effect of guanine nucleotides on stimulation of human myocardial PLC by mastoparan

	Inositol phosphate formation (nmol min^{-1} mg^{-1} protein)
Control	11.8 ± 0.3
GDP	11.3 ± 0.7
GDPβS	11.0 ± 0.4
Mas	21.7 ± 0.6
Mas + GDP	20.6 ± 1.0
Mas + GDPβS	22.1 ± 1.3

Preparations from human myocardial membranes (1 μg protein/tube) were incubated with phospholipid vesicles containing PtdInsP_2 for 30 min at 25°C at 1 μM Ca^{2+} under control conditions, in the presence of GDP (100 μM), GDPβS (100 μM), mastoparan (10 μM), mastoparan in combination with GDP and mastoparan in combination with GDPβS. The values correspond to means $\pm$ s.d. of triplicate determinations. The experiments shown are representative of three experiments performed.

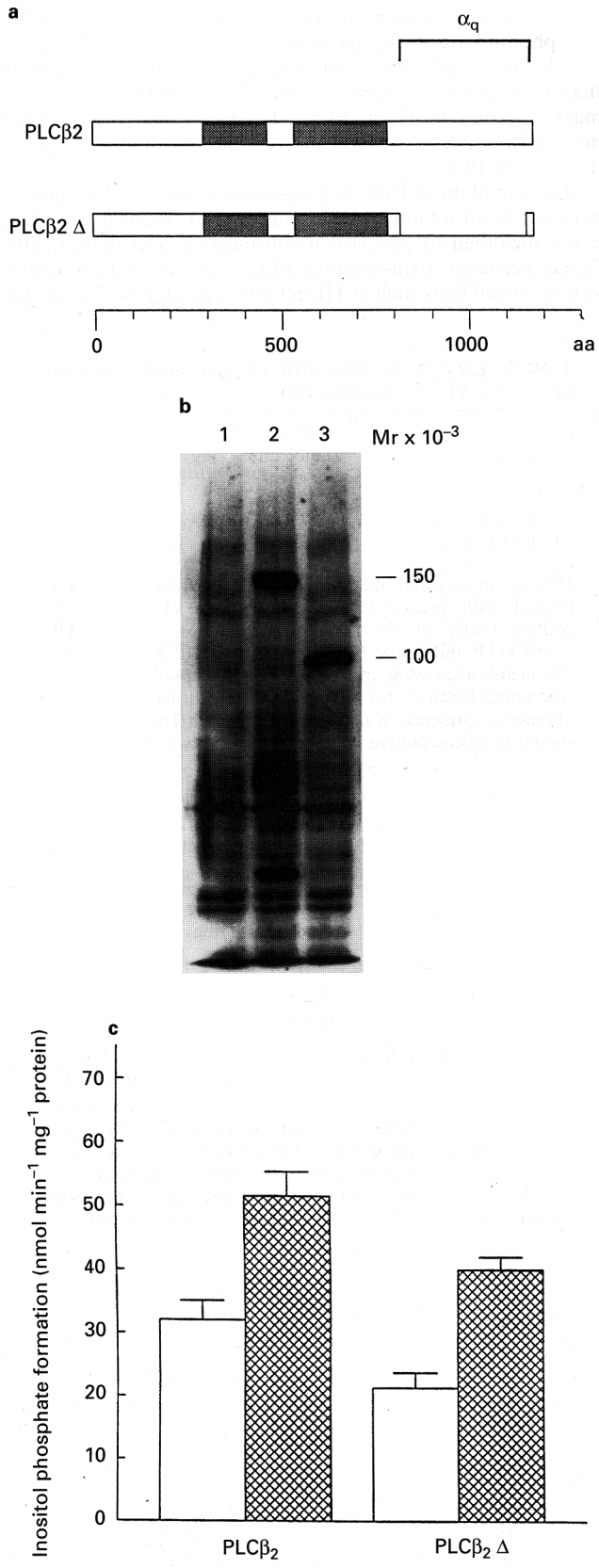

Figure 2 (a) Schematic representation of wild-type PLCβ_2 and PLC$\beta_2\Delta$ (PLC$\beta_2\Delta$[F819-E1166]). The region deleted in PLC$\beta_2\Delta$ contains the site of interaction with α_q. The shaded areas represent the two domains of high sequence homology which are found in all PLC isozymes known. The amino acid (aa) numbering is given below. (b) Immunochemical analysis of PLCβ_2 and PLC$\beta_2\Delta$. COS-1 cells were transfected with the expression plasmid pMT2 without insert (lane 1), or pMT2 containing the cDNA of PLCβ_2 (lane 2; $\approx$150 kDa) and PLC$\beta_2\Delta$ (lane 3; $\approx$100 kDa). Detergent extracts of transfected cells were subjected to SDS/PAGE (150 μg protein/lane) and Western blotting was performed with antibodies raised against the carboxy terminal pentadecapeptide of PLCβ_2. Immunoreactive proteins were visualized by chemoluminescence as described in Methods. The positions of the molecular-mass standards are indicated. (c) Stimulation of PLCβ_2 and PLC$\beta_2\Delta$ by mastoparan. Detergent extracts (0.3 μg protein/tube) of COS-1 cells expressing PLCβ_2 and PLC$\beta_2\Delta$, respectively, were incubated with phospholipid vesicles containing $PtdInsP_2$ for 30 min at 25°C in the absence and presence of mastoparan (10 μM) at 1 μM free calcium. The values correspond to means$\pm$s.d. of triplicate determinations. The experiment shown is representative of three experiments performed. The background of COS-1 cell PLC was less than 5% of PLC activity measured in the lysates shown.

to G protein $\beta\gamma$-subunits (Camps *et al*., 1992b), the question of whether $\beta\gamma$-subunits mediate mastoparan stimulation was addressed directly (Table 3). PLC activation by mastoparan was not abolished by 4 μM α_t, a scavenger of free $\beta\gamma$-subunits, suggesting the mastoparan effect is not due to stimulation of G protein $\beta\gamma$-subunits. As a control for the functionality of purified α_t, PLC stimulation by purified exogenous $\beta\gamma$-subunits was reversed by addition of a two fold excess of α_t (not shown). As shown in Table 4, neither GDP nor guanosine-5′*O*-(β-thiodiphosphate) (GDPβS) were able to prevent mastoparan stimulation of myocardial PLC, although GDP has previously been shown to abolish G protein-dependent stimulation by GTPγS (Schnabel *et al*., 1996). These results argue against the involvement of G proteins in the mediation of the mastoparan effect observed.

In order to clarify whether α_q proteins are involved in mastoparan stimulation of PLC enzymes, a deletion mutant of PLCβ_2 lacking the site of interaction of the enzyme with α_q (Schnabel *et al*., 1993; Park *et al*., 1993; Wu *et al*., 1993a, b) was constructed. Although the predominant PLCβ isozyme present in human myocardium is PLCβ_1, the deletion mutant was derived from PLCβ_2 for methodological reasons. However, the sites of interaction of PLCβ_1 and PLCβ_2, respectively, are located at corresponding regions of the enzymes (Schnabel *et al*., 1993; Park *et al*., 1993; Wu *et al*., 1993a, b). COS-1 cells were transiently transfected with the wild-type and mutant PLCβ_2 cDNA (Figure 2a). Expression was verified by immunoblotting with rabbit antibodies reactive against the carboxyl-terminal pentadecapeptide of PLCβ_2 (Figure 2b). As a negative control, mock-transfected cells were investigated and exhibited no immunoreactive band in the Western blot (Figure 2b). The deletion mutant (PLC$\beta_2\Delta$) was stimulated by mastoparan to a similar extent as the wild-type enzyme (Figure 2c), indicating that this effect is not mediated by α_q subunits. PLC activity in the control lysates was less than 5% of the activity in lysates from transfected cells, indicating that the activity measured corresponded to recombinant PLC enzymes and not to the background activity present in COS-1 cells.

Taken together, stimulation of human myocardial PLC by mastoparan is most likely due to either a direct interaction of the peptide with the enzyme or to a mechanism involving mediators distinct from heterotrimeric G proteins.

Discussion

In the present study, we have provided evidence for a sequence-specific and G protein independent stimulation of human myocardial PLC by the wasp venom peptide mastoparan.

Mastoparan is able to stimulate heterotrimeric G proteins by promoting the GDP/GTP exchange at the α-subunit (Higashijima *et al*., 1988; 1990), by a mechanism similar to that of G protein-coupled receptors. Evidence for a direct interaction of mastoparan with G protein α-subunits has been provided by crosslinking of iodinated mastoparan to the amino-terminal region of α_o (Higashijima & Ross, 1991). Mastoparan exerts the strongest effects on the pertussis toxin-sensitive G proteins G_i and G_o, but has been shown to interact with pertussis toxin-

insensitive G proteins as well, albeit with a lower efficiency (Ross & Higashijima, 1994). Thus it is not surprising that mastoparan stimulates PLC in certain cellular systems like rat mast cells (Mousli *et al*., 1989), rat hepatocytes (Tohkin *et al*., 1990; Garcia-Sainz *et al*., 1991), human HL-60 leukaemia cells (Gusovski *et al*., 1991; Norgauer *et al*., 1992), and rat PC-12 phaeochromocytoma cells (Choi *et al*., 1992). In contrast to these findings, inhibition of PLC by mastoparan has been described in human SH-SY5Y neuroblastoma (Wojcikiewcz & Nahorski, 1989) and astrocytoma (Nakahata *et al*., 1990) cells. In the membrane preparation used for the assessment of PLC activity in the present study, there was a marked and reproducible stimulation of the enzyme by mastoparan. This could be due to (i) activation of heterotrimeric G proteins, (ii) interaction with the phosphlipid substrate, (iii) direct interaction with the PLC molecule, or (iv) mediation by factors other than heterotrimeric G proteins. In our system, mediation of the mastoparan effect by heterotrimeric G proteins appears unlikely for several reasons: firstly, the PLC stimulation was pertussis toxin-insensitive, repudiating the involvement of G_i and G_o, the proteins on which mastoparan is most effective. The pertussis toxin-resistance of the effect provides indirect evidence against an involvement of $\beta\gamma$-subunits as G_i proteins are highly abundant in the human heart and could theoretically release enough $\beta\gamma$-subunits to activate PLC. More direct evidence against a $\beta\gamma$-mediated effect is provided by the control experiment showing that mastoparan stimulation is not abolished by addition of an excess of GDP-bound α_t, which is a well-established scavenger of free $\beta\gamma$-subunits (Camps *et al*., 1992a, b; Schnabel *et al*., 1996). A third line of evidence refuting the involvement of heterotrimeric G proteins in mastoparan stimulation of myocardial PLC is provided by the observation that the mastoparan effect is also present in the cytosolic fraction of human myocardium where G_i and G_q proteins are not present, as assessed by Western blotting (not shown). Moreover, PLC stimulation by mastoparan was not abolished by addition of GDP or GDPβS. However, one could still argue that the lack of a GDP effect does not exclude the involvement of α_q in PLC-stimulation by mastoparan, as the guanine nucleotide turnover of G_q proteins is lower than that of most other G proteins known (Pang & Sternweis, 1990). PLCβ isozymes are stimulated by α_q through a protein-protein interaction in the carboxyl-terminal region of the molecule. Removal of most of the amino acid residues downstream of the putative catalytic domain Y either by limited proteolysis (Park *et al*., 1993) or by site-directed mutagenesis (Wu *et al*., 1993a, b; Schnabel *et al*., 1993) results in a preserved catalytic and $\beta\gamma$-subunit-stimulated activity, but in a loss of α_q sensitivity. PLCβ_1, PLCβ_2 and PLCβ_3 are all sensitive to stimulation by α_q- as well as $\beta\gamma$-subunits (Smrcka *et al*., 1993). Although PLCβ_2 is not present in human myocardium (Schnabel *et al*., 1996), but evidence for PLCβ_1 expression has been provided, it is reasonable to assume that a deletion mutant of PLCβ_2 deficient of the carboxyl-terminal region of the molecule is an appropriate control for α_q interaction with PLCβ isozymes.

Wallace & Carter (1989) observed that PLCβ_1 purified from bovine brain was stimulated by mastoparan through an interaction of the peptide with the substrate. The PLC stimulation by mastoparan correlated with the ability of the peptide to restrict aggregation of $PtdInsP_2$ into higher order structures. Another study providing evidence for stimulation of PLCβ enzymes by interaction of amphiphilic peptides with the phospholipid substrate was presented recently (Simoes *et al*., 1995b). In this work, a basic peptide previously shown to stimulate PLCβ_2 (Simoes *et al*., 1993) was demonstrated to change its conformation by interaction with $PtdInsP_2$. Moreover, a number of polyamines and basic proteins are capable of stimulating PLCδ purified from rat liver by either interaction of the peptides with the substrate or with the enzyme or both (Haber *et al*., 1991). Evidence for a direct interaction of mastoparan with PLCβ_1, in addition to the interaction of the peptide with $PtdInsP_2$, was provided by the binding of the enzyme to a mastoparan sepharose column (Wallace & Carter, 1989). In our experiments, the stimulant effect of mastoparan on PLC is unlikely to be due merely to the amphiphilic properties of the peptide, as Mas17, whose amphiphilicity is similar to that of mastoparan (Ross & Higashijima, 1994), was inactive. Thus the effect of mastoparan appears to be sequence-specific, but it could still be a sequence-specific interaction with the substrate or with the enzyme. The immobilization of mastoparan or purified PLCβ as well as crosslinking experiments might elucidate this issue in the future.

The mechanism of PLCβ stimulation by mastoparan could also involve molecules distinct from heterotrimeric G proteins. Mastoparan has previously been shown to activate *rho* and *rac*, two small molecular weight GTP-binding proteins (Koch *et al*., 1991). These proteins are potential signal transducers to PLC. The idea of an interaction of small molecular weight GTP-binding proteins with PLCβ enzymes is supported by the recent findings of Malek & Gierschik (1995) that small molecular weight GTP-binding proteins of the *rho* family stimulate PLCβ_2. Another potential mediator of mastoparan activation of G proteins is nucleoside diphosphate kinase (NDPK), an enzyme which generates GTP and has recently been shown to be stimulated by mastoparan (Klinker *et al*., 1996). Further research will be necessary to characterize the role of these signalling molecules in physiological responses such as growth and differentiation. Mastoparan might be a useful tool for this purpose.

We thank Dr Peter Parker, Imperial Cancer Research Fund (London, U.K.) for providing the PLCβ_2 cDNA and antibody. We are also grateful to Katja Moosdorf for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (Schn 517/1-1) and the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (01 KS 9502).

References

BERRIDGE, M.J. (1993). Inositol triphosphate and calcium signalling. *Nature*, **361,** 315 – 325.

CAMPS, M., CAROZZI, A., SCHNABEL, P., SCHEER, A., PARKER, P.J. & GIERSCHIK, P. (1992a). Isozyme-selective stimulation of phospholipase C-β_2 by G protein $\beta\gamma$-subunits. *Nature*, **360,** 684 – 686.

CAMPS, M., HOU, C., SIDIROPOULOS, D., STOCK, J.B., JAKOBS, K.H. & GIERSCHIK, P. (1992b). Stimulation of phospholipase C by guanine-nucleotide-binding protein $\beta\gamma$ subunits. *Eur. J. Biochem.*, **206,** 821 – 831.

CHOI, O.H., PADGETT, W.L. & DALY, J.W. (1992). Effects of the amphiphilic peptides melittin and mastoparan on calcium influx, phosphoinositide breakdown and arachidonic acid release in rat pheochromocytoma PC12 cells. *J. Pharmacol. Exp. Ther.*, **260,** 369 – 375.

GARCIA-SAINZ, J.A., MACIAS-SILVA, M., & ROMERO-AVILA, M.T. (1991). Effect of okadaic acid on hormone- and mastoparan-stimulated phosphoinositide turnover in isolated rat hepatocytes. *Biochem. Biophys. Res. Commun.*, **179,** 852 – 858.

GUSTOVSKY, F., SOERGEL, D.G. & DALY, J.W. (1991). Effects of mastoparan and related peptides on phosphoinositide breakdown in HL-60 and cell-free preparations. *Eur. J. Pharmacol.*, **206,** 309 – 314.

HABER, M.T., FUKUI, T., LEBOWITZ, M.S. & LOWENSTEIN, J.M. (1991). Activation of phosphoinositide-specific phospholipase Cδ from rat liver by polyamines and basic proteins. *Arch. Biochem. Biophys.*, **288,** 243 – 249.

HIGASHIJIMA, T., BURNIER, J. & ROSS, E.M. (1990). Regulation of Gi and Go by mastoparan, related amphiphilic peptides, and hydrophobic amines. *J. Biol. Chem.*, **265,** 14176 – 14186.

HIGASHIJIMA, T. & ROSS, E.M. (1991). Mapping of the mastoparan-binding site on G proteins. *J. Biol. Chem.*, **266,** 12655 – 12661.

HIGASHIJIMA, T., UZU, S., NAKAJIMA, T. & ROSS, E.M. (1988). Mastoparan, a peptide toxin from wasp venom, mimics receptors by activating GTP-binding regulatory proteins (G proteins). *J. Biol. Chem.*, **263,** 6491 – 6494.

KATZ, A., WU, D. & SIMON, M.I. (1992). Subunits $\beta\gamma$ of heterotrimeric G protein activate β_2 isoform of phospholipase C. *Nature*, **360,** 686 – 689.

KIM, M.J., BAHK, Y.Y., MIN, D.S., LEE, S.J., RYU, S.H. & SUH, P.G. (1993). Cloning of cDNA encoding rat phospholipase C-β_4, a new member of the phospholipase C. *Biochem. Biophys. Res. Commun.*, **194,** 706 – 712.

KLINKER, J.F., LAUGWITZ, K.L. HAGELÜKEN, A. & SEIFERT, R. (1996). Activation of GTP formation and high affinity GTP hydrolysis of mastoparan in various cell membranes. G-protein activation via nucleoside diphosphate kinase, a possible general mechanism of mastoparan action. *Biochem. Pharmacol.*, **51,** 217 – 223.

KOCH, G., HABERMANN, B., MOHR, C., JUST, I. & AKTORIES, K. (1991). Interaction of mastoparan with the low molecular mass GTP-binding protein rho/rac. *FEBS Lett.*, **291,** 336 – 340.

LEE, C.W., PARK, D.J., LEE, K.H., KIM, C.D. & RHEE, S.G. (1993). Purification, molecular cloning, and sequencing of phospholipase C-β_4. *J. Biol. Chem.*, **268,** 21318 – 21327.

LEE, Y.J., KANG, S.I., AHN, D.S., LEE, H.Y., CHOI, E.J. & KANG, B.S. (1995). Effects of mastoparan on a vascular contractility in rabbit aorta. *Yonsei Med. J.*, **36,** 262 – 270.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDAL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193,** 265 – 275.

MALEK, D. & GIERSCHIK, P. (1995). Purification from bovine neutrophils and characterization of a cytosolic factor mediating guanosine 5′[γ-thio]triphosphate stimulation of phospholipase C-β_2. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **351,** (Suppl.), 263 (abstract).

MOUSLI, M., BRONNER, C., BUEB, J.L., TSCHIRHART, E., GIES, J.P. & LANDRY, Y. (1989). Activation of rat peritoneal mast cells by substance P and mastoparan. *J. Pharmacol. Exp. Ther.*, **250,** 329 – 335.

NAKAHATA, N., ABE, M.T., MATSUOKA, I. & NAKANISHI, H. (1990). Mastoparan inhibits phosphoinositide hydrolysis via pertussis toxin-sensitive G-protein in human astrocytoma cells. *FEBS Lett.*, **260,** 91 – 94.

NISHIZUKA, Y. (1992). Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science*, **258,** 607 – 614.

NORGAUER, J., EBERLE, M., LEMKE, H.D. & AKTORIES, K. (1992). Activation of human neutrophils by mastoparan. Reorganization of the cytoskeleton, formation of phosphatidylinositol 3,4,5-trisphosphate, secretion, up-regulation of complement receptor type 3 and superoxide anion production are stimulated by mastoparan. *Biochem. J.*, **282,** 393 – 397.

PANG, I.H. & STERNWEIS, P.C. (1990). Purification of unique α subunits of GTP-binding proteins (G proteins) by affinity chromatography with immobilized $\beta\gamma$ subunits. *J. Biol. Chem.*, **265,** 18707 – 18712.

PARK, D., JHON, D.Y., LEE, C.W., LEE, K.H. & RHEE, S.G. (1993). Activation of phospholipase C isozymes by G protein $\beta\gamma$ subunits. *J. Biol. Chem.*, **268,** 4573 – 4576.

RHEE, S.G. & CHOI, K.D. (1992). Regulation of inositol phospholipid-specific phospholipase C isozymes. *J. Biol. Chem.*, **267,** 12393 – 12396.

ROSS, E.M. & HIGASHIJIMA, T. (1994). Regulation of G-protein activation by mastoparans and other cationic peptides. *Methods Enzymol.*, **237,** 26 – 37.

SCHNABEL, P., CAMPS, M., CAROZZI, A., PARKER, P.J. & GIERSCHIK, P. (1993). Mutational analysis of phospholipase C-β_2: Identification of regions required for membrane association and stimulation by G-protein $\beta\gamma$ subunits. *Eur. J. Biochem.*, **217,** 1109 – 1115.

SCHNABEL, P., GÄS, H., NOHR, T., CAMPS, M. & BÖHM, M. (1996). Identification and characterization of G protein-regulated phospholipase C in human myocardium. *J. Mol. Cell. Cardiol.*, **28,** 2419 – 2426.

SIMOES, A.P., REED, J., SCHNABEL, P., CAMPS, M. & GIERSCHIK, P. (1995b). Characterization of putative polyphosphoinositide binding motif from phospholipase C-β_2. *Biochemistry*, **34,** 5113 – 5119.

SIMOES, A.P., SCHNABEL, P., PIPKORN, R., CAMPS, M. & GIERSCHIK, P. (1993). A peptide corresponding to a potential polyphosphoinositide binding site of phospholipase C-β_2 enhances its catalytic activity. *FEBS Lett.*, **331,** 248 – 251.

SIMPSON, P.C. (1983). Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an alpha 1 adrenergic response. *J. Clin. Invest.*, **72,** 732 – 738.

SMRCKA, A.V. & STERNWEIS, P.C. (1993). Regulation of purified subtypes of phosphatidylinositol specific phospholipase C β by G protein α and $\beta\gamma$ subunits. *J. Biol. Chem.*, **268,** 9667 – 9674.

TERZIC, A., PUCEAT, M., VASSORT, G. & VOGEL, S.M. (1993). Cardiac α_1-adrenoceptors: An overview. *Pharmacol. Rev.*, **45,** 147 – 175.

TOHKIN, M., YAGAMI, T. & MATSUBARA, T. (1990). 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. *FEBS Lett.*, **260,** 179 – 182.

WALLACE, M.A. & CARTER, H.R. (1989). Effects of the wasp venom peptide, mastoparan, on a phosphoinositide-specific phospholipase C purified from rabbit brain membranes. *Biochim. Biophys. Acta*, **1006,** 311 – 316.

WOJCIKIEWICZ, R.J.H. & NAHORSKI, S.R. (1989). Phosphoinositide hydrolysis in permeabilized SH-SY5Y human neuroblastoma cells is inhibited by mastoparan. *FEBS Lett.*, **247,** 341 – 344.

WU, D., JIANG, H., KATZ, A. & SIMON, M.I. (1993a). Identification of critical regions on phospholipase C β_1 required for activation by G proteins. *J. Biol. Chem.*, **268,** 3704 – 3709.

WU, D., KATZ, A. & SIMON, M.I. (1993b). Activation of phospholipase C β_2 by the α and $\beta\gamma$ subunits of trimeric G protein. *Proc. Natl. Acad. Sci. U.S.A.*, **90,** 5297 – 5301.

(Received February 14, 1997
Revised May 12, 1997
Accepted May 30, 1997)