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Direct regulation of vascular smooth muscle contraction by mastoparan-7

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Abstract. Mastoparan-7 (mas-7) is a basic tetradecapeptide isolated from wasp venom, which activates guanine nucleotide-binding regulatory proteins (G-proteins) and stimulates apoptosis. In smooth muscle cells, mas-7 leads to an increase in the perfusion pressure. The main aim of this study was to evaluate the physiological effect of the direct stimulation of G-proteins in comparison to the typical stimulation of receptors in vascular smooth muscle cells (VSMCs). Experiments were performed on the isolated and perfused tail artery of Wistar rats. The contraction force in our model was measured by an increased level of perfusion pressure with a constant flow. The concentration response curves (CRCs) obtained for mas-7 were sigmoidal. In comparison to the curves for phenylephrine and vasopressin, the mas-7 curve was significantly shifted to the right with a significant reduction in maximal response. Mas-7 significantly increased the perfusion pressure for the intra- and extracellular calcium (Ca^{2+}) influx to the cytoplasm. The presence of the pertussis toxin (PT) did not affect the mas-7-induced contraction. In comparison to phenylephrine and vasopressin, all the values of perfusion pressure following stimulation of the G-proteins by mas-7 were significantly lower. The results of our experiments suggested that mas-7 significantly induces the contraction of VSMCs. The binding site for mas-7 is different from that for PT; thus, PT does not affect VSMC contraction. The tissue effect of this stimulation is comparable to the stimulatory effect of partial agonists. Current knowledge regarding the apoptosis pathway reveals the significance of Ca^{2+} ions involved in this process. Therefore, mas-7 may induce apoptosis through an increase in the cytoplasmic Ca^{2+} concentration; however, the use of this mechanism in anticancer therapy must be preceded by a molecule modification that eliminates the vasoconstrictive effect.

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Abbreviations: CRC, concentration response curve; EC_{50}, half maximal effect concentration; E_{max}, maximal tissue response; mas-7, mastoparan-7; PLC, phospholipase C; PT, pertussis toxin

Key words: mastoparan-7, mastoparan-17, phospholipase C, G-protein

Introduction

Mastoparan-7 (mas-7) is a basic tetradecapeptide isolated from wasp venom, which activates guanine nucleotide-binding regulatory proteins (G-proteins) by catalyzing the guanosine 5'-diphosphate/guanosine 5'-triphosphate (GDP/GTP) exchange. Thus, this compound mimics the action of activated G-protein-coupled receptors. Mas-7 has been shown to stimulate phospholipase C (PLC) in several cellular compartments, such as rat mast cells, rat hepatocytes and HL-60 human leukaemia cells. By contrast, the inhibition of PLC by mastoparan has been demonstrated in SH-SY5Y human neuroblastoma cells and in human astrocytoma cells (1). Recent studies suggested the possibility of programmed cell death stimulation in different types of cells (2-4).

The pertussis toxin (PT) catalyzes the adenosine 5'-diphosphate-ribosylation of the α subunits of the heterotrimeric G_i, G_o and G_t proteins. This prevents the G-protein heterotrimers from interacting with their receptors, thus blocking their coupling and activation. Since the G_α subunits remain in their GDP-bound, inactive state, they are unable to inactivate adenylyl cyclase or open K^+ channels (5). PT is commonly used in several models of signaling pathways.

Calcium (Ca^{2+}) ions play a central role in the life of the cell. Accordingly to pathological factors, Ca^{2+} concentration changes occur in various cell compartments, which may induce apoptosis. Prolonged Ca^{2+} ions concentration changes in the cytoplasm, nucleus or mitochondria, may initiate the cascade of events that lead to cell death. Following stimulation by pathological factors, Ca^{2+} ions are released from the endoplasmic reticulum and bind to several molecules, such as calpain or calcineurin. Calpain belongs to the cysteine protease family, which activates BH3 interacting-domain death agonist and Bcl-2-associated X protein and it promotes their transport to the mitochondria. In addition, Ca^{2+} excess in the mitochondria leads to release of proapoptotic proteins located in the intracellular space, such as the second mitochondria-derived activator of caspases/Diablo and cytochrome *c* (6,7).

Table I. Half maximal effect concentration (EC_{50}), maximal tissue response (EC_{max}) and relative potency (RP) for mas-7 for controls and in the presence of PT.

Compound	n[a]	$\%E_{max}$[b]	EC_{50} (M/l)	pD_2[e]	RP[c]	P-value[d]
Mas-7	16	37±4	4.41 (±2.33) $x10^{-8}$	7.40±0.20	1.000	-
Mas-7+PT	11	35±4	6.12 (±3.40) $x10^{-8}$	7.21±0.22	0.721	0.1593
Phenylephrine	34	100	7,51 (±0,97) $x10^{-8}$	7,13±0,06	-	<0.0001
Vasopressin	25	100	1,82 (±0,61) $x10^{-8}$	7,76±0,14	-	<0.0001

[a]Number of concentration-response curves used for the calculations; [b]E_{max} calculated as a percentage of maximal response for controls; [c]calculated as EC_{50} for controls/EC_{50}; [d]P-value calculated in comparison to control values; [e]negative logarithm of the EC_{50}. Mas-7, mastoparan-7; PT, pertussis toxin.

The main aim of this study was to evaluate the physiological effect of the direct stimulation of the G-proteins in comparison to the typical stimulation of α-adrenergic receptors and vasopressin receptor type 1 in vascular smooth muscle cells (VSMCs).

Materials and methods

Animals. The experiments were performed on the isolated and perfused tail artery of Wistar rats (weight, 250-270 g). The animals were housed under a 12-h light/dark cycle and had unlimited access to food and water. The rats were narcotized by intraperitoneal injection of 120 mg urethane per 1 kg body weight and were sacrificed by stunning and cervical dislocation. The study protocol was approved by the local Ethics Committee. All the studies were performed in accordance with the United States NIH guidelines [Guide for the Care and Use of Laboratory Animals (1985), DHEW Publication No. (NIH) 85-23: Office of Science and Health Reports, DRR/NIH, Bethesda, MD, USA].

Drugs and solutions. Mas-7 was used as a G-protein activator and mastoparan-17 (mas-17) was used as negative control. The Krebs solution consisted of NaCl (71.8 mM/l), KCl (4.7 mM/l), $CaCl_2$ (1.7 mM/l) $NaHCO_3$ (28.4 mM/l), $MgSO_4$ (2.4 mM/l), KH_2PO_4 (1.2 mM/l) and glucose (11.1 mM/l). All the reagents were purchased from Sigma-Aldrich Chemical Co. (Poznań, Poland). Study design and conduction. Following dissection from the surrounding tissues, a 2-3-cm long segment of a rat tail artery was cannulated and connected to a perfusion device. The distal part was weighted with a 500-mg weight and the tail was placed in a 20-ml container filled with oxygenated Krebs solution at 37˚C (pH 7.4). The perfusion pressure was continuously measured. The perfusion solution flow was gradually increased using a peristaltic pump to 1 ml/min, until the optimum perfusion pressure of 2-4 kPa (8,9).

Data analysis and statistical procedures. The investigations were performed on the TSZ-04 system (Experimetria, Ltd., Balatonfüred, Hungary). The perfusion pressure was measured on BPR-01 and BPR-02 devices and the vascular smooth muscle tension was measured on an FSG-01 transducer. All the transducers used in our experiments were provided by Experimetria, Ltd. The peristaltic pump was provided by Zalimp, Warsaw, Poland.

The concentration response curves (CRCs) were calculated according to the van Rossum method. The maximum response of the tissue (E_{max}) was calculated as the percentage of the maximal response for phenylephrine. The half maximal effective concentration (EC_{50}) was estimated using classical pharmacological methods with pD2, the negative logarithm of the EC_{50}. We used CRC and E_{max} in all the calculations estimating statistical significance. Mas-17 was used as negative control.

The results are presented as mean values ± standard deviation. The statistical analysis was performed using the analysis of variance for multiple comparisons of the means. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Mas-7 CRCs. The CRCs obtained for mas-7 were sigmoidal. In comparison to the curves for phenylephrine and vasopressin, the mas-7 curve was shifted to the right with a significant reduction in maximal response (Fig. 1). For all the points for a relative effect of ≥20%, the differences were statistically significant. The curve obtained for mas-7 in the presence of PT did not differ significantly from the control. The calculated E_{max}, EC_{50} and pD2 values are presented in Table I.

Effect of G-protein activation by mas-7 on perfusion pressure. Analyzing the perfusion pressure as a result of the contraction induced by Ca^{2+} influx from the intracellular Ca^{2+} stores with mas-7 (phase 1), a significant increase was observed in comparison to the negative control mas-17. The same association was observed following mas-7-induced extracellular Ca^{2+} influx to the cytoplasm (phase 2). The presence of PT did not significantly affect the mas-7-induced contraction. In comparison to phenylephrine and vasopressin, all the values of perfusion pressure following stimulation of the G-proteins by mas-7 were significantly lower (Fig. 2, Table II).

Discussion

In the performed experiment, vascular contraction was induced by mas-7, an activator of G-proteins. The vasoconstriction triggered by mas-7 exhibited a slower increase compared to that

Table II. Maximal perfusion pressure during mas-7-induced contraction activated by Ca^{2+} influx from intracellular (phase 1) and extracellular (phase 2) Ca^{2+} stores.

	Intracellular Ca^{2+} (phase 1)		Extracellular Ca^{2+} (phase 2)	
Compound	n[d]	Perfusion pressure (±SE) (mm/Hg)	n	Perfusion pressure (±SE) (mm/Hg)
Mas-17	10	11.8 (±2.1)	10	10.1 (±2.4)
Mas-7	16	17.4 (±3.1)[a,b]	16	28.3 (±5.6)[a,b]
Mas-7+PT	11	18.2 (±4.7)[a,c]	11	27.4 (±8.5)[a,c]
Phenylephrine	30	57.9 (±7.2)	30	93.6 (±7.8)
Vasopressin	32	62.4 (±6.4)	32	103.2 (±6.0)

[a]P<0.0001 vs. mas-17; [b]P<0.0001 vs. phenylephrine and vasopressin; [c]non-significant vs. mas-7; [d]number of concentration-response curves used for the calculations. PT, pertussis toxin; mas, mastoparan; SE, standard error.

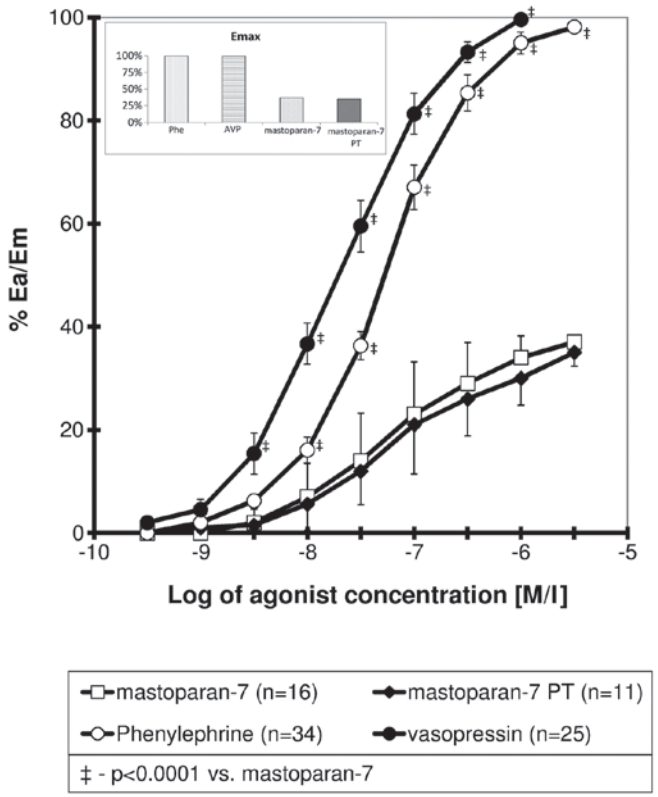

Figure 1. Concentration response curves for mastoparan-7 in the control and in the presence of pertussis toxin (PT).

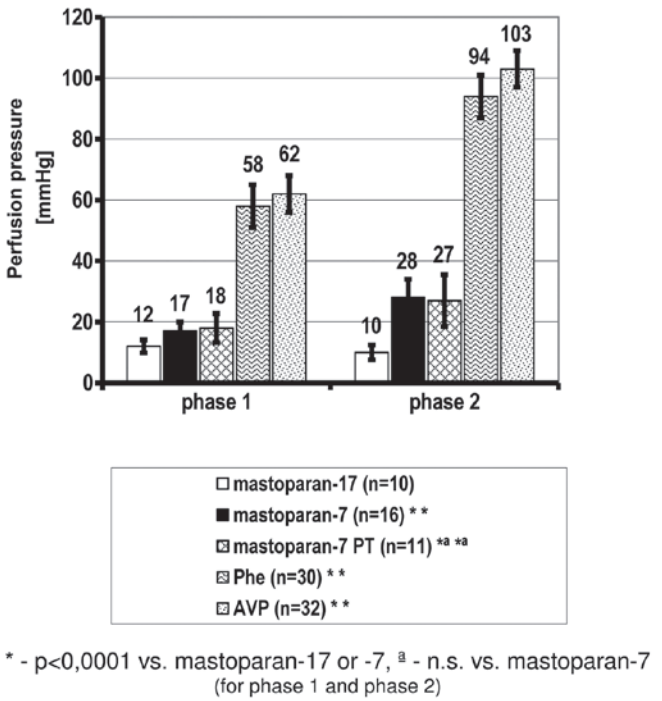

Figure 2. Effect of G-protein activation by mastoparan-7 on perfusion pressure triggered with intra- and extracellular Ca^{2+} pool in comparison to the control (mastoparan-17) and in the presence of pertussis toxin (PT). Phe, phenylephrine; AVP, vasopressin; n.s., non-significant.

simulated by phenylephrine or vasopressin. In response to the stimulation of α_1-adrenergic, vasopressin or angiotensin receptors, vasoconstriction was observed within a few seconds, whereas the maximum response to mas-7 appeared after 30-40 min. The present study demonstrated that PT exerted no inhibitory effect on the vasoconstriction stimulated by mas-7. Kanagy and Webb (10) previously investigated spiral cutting fragments of the common carotid artery and demonstrated a measureable response after 10 min and a maximum response after 30 min following the application of mas-7 (10^{-5} M/l) (11). Furthermore, in rats with hypertension, the arterial reactivity was significantly higher compared to that of controls. PT and the phospholipase A_2 inhibitor indomethacin did not affect the response to mas-7. Nifedipine at a concentration of 10^{-5} M/l was shown to inhibit the contraction of VSMCs induced by mas-7 at a concentration of 10^{-7} M/l, revealing a correlation between voltage-dependent Ca^{2+} channels and mas-7-induced vasoconstriction. The lack of complete reversal by nifedipine at higher concentrations of mas-7 (10^{-5} M/l) suggests that an additional mechanism may be activated at higher concentrations (10).

In this study, the effect of the G-protein inhibitor PT on mas-7-induced contraction was investigated. We observed a notable inhibition of VSMC contraction triggered by G-protein activation and a proportional perfusion pressure reduction caused by intra- and extracellular Ca^{2+} influx.

The vasoconstriction induced by the activation of metabotropic receptors, such as α-adrenergic receptors, vassopresin receptors (V_1) or angiotensin II receptors type 1, is conditioned upon the activation of G-proteins. Subsequently, G-proteins activate PLC, leading to the the hydrolysis of phosphatidylinositol 4,5-bisphosphate and increased intracellular concentration of inositol-1,4,5-triphosphate (11-13). Mas-7 penetrates through biological barriers and binds to the ligand-binding site of the G-protein-coupled receptor, stimulating G-proteins in a way similar to an activating receptor. As demonstrated by biochemical studies, the affinity of mastoparan for individual G-protein types is significantly different. Mas-7 exhibits a higher affinity for G_i and G_o compared to the G_s protein (14). PT does not affect the VSMC contraction induced by mas-7 by inhibiting the function of G_i and G_o, indicating that the target in this process may be G_q. Secondarily to the activation of G_q and PLC, the metabolism of membrane phospholipids may be increased (10). Ca^{2+} channel blockers directly inhibit Ca^{2+} influx, thereby decreasing the efficiency of the contraction induced by mas-7, highlighting the role of Ca^{2+} channels in this process (8,10,15,16). A previous study conducted by Perianin and Snyderman (15) demonstrated that mas-7 may increase Ca^{2+} ion concentration in the cytoplasm through mechanisms which are unrelated to the production of inositol triphosphate and diacylglycerol.

The affinity for the G_q-protein has not been specified thus far; however, functional investigations were performed on the process of G-protein activation with mas-7 in the VSMCs of the carotid artery in rats (10). The results demonstrated that mas-7 activates G_q-proteins in VSMCs, leading to the increase of Ca^{2+} ion concentration in the cytoplasm and resulting vasoconstriction. Moreover, in rats with genetically determined hypertension, the contraction of VSMCs was significantly more prominent compared to the control group (10). Mas-7 may activate phospolipase A_2 at a concentration of $5x10^{-5}$ M/l, leading to the degranulation of mast cells (17). In VSMCs, the process of prostanoid production does not modify the contraction triggered by mas-7, as determined by experiments performed in the presence of indomethacin. No significant effect of indomethacin was demonstrated in those studies (10,15). Mas-7 at a concentration of 10^{-5} M/l may affect vasoconstriction through additional mechanisms, such as Ca^{2+} channel modulation and voltage-independent Ca^{2+} channels (15). Mas-7 also exerts a direct effect on PLC. At low concentrations (<$3x10^{-6}$ M/l), PLC activation was inhibited by mas-7, although at higher concentrations (>$5x10^{-6}$ M/l) direct activation was observed (18,19). In our study, a lower concentration of mas-7 ($3x10^{-10}$-10^{-6} M/l) was used, which was not sufficiently high to affect elements of signaling pathway other than the G-proteins. The vasoconstriction induced by mas-7 depends on the intra- and extracellular Ca^{2+} pool, which may also affect apoptosis. The results of this process were higher values of perfusion pressure.

The constriction of VSMCs induced by mas-7 was significantly lower in comparison to that induced by phenylephrine and vasopressin, as was previously confirmed by Kanagy and Webb (10). The effects observed for mas-7 were similar to the effect observed following activation by partial receptor agonists, rather than full agonists such as phenylephrine or vasopressin. Similar perfusion pressure values were reported with the α_2-receptor agonist clonidine (20).

In conclusion, our results suggest that mas-7 significantly induces VSMC contraction. The binding site for mas-7 is different from that for PT; therefore, PT does not affect VSMC contraction. The tissue effect of this stimulation is comparable to the effect of stimulation with partial receptor agonists. Our current knowledge regarding the apoptosis pathway demonstrates the significance of Ca^{2+} ions involved in this process. Thus, mas-7 may induce apoptosis through an increase in the cytoplasmic Ca^{2+} concentration; however, the use of this mechanism in anticancer therapy must be preceded by a molecule modification that eliminates the vasoconstrictive effect.

References

1. King TP, Jim SY and Wittkowski KM: Inflammatory role of two venom components of yellow jackets (*Vespula vulgaris*): a mast cell degranulating peptide mastoparan and phospholipase A1. Int Arch Allergy Immunol 131: 25-32, 2003.
2. Hoshina MM, Santos LD, Palma MS and Marin-Morales MA: Cytotoxic, genotoxic/antigenotoxic and mutagenic/antimutagenic effects of the venom of the wasp *Polybia paulista*. Toxicon 72: 64-70, 2013.
3. Yordanova ZP, Woltering EJ, Kapchina-Toteva VM and Iakimova ET: Mastoparan-induced programmed cell death in the unicellular alga *Chlamydomonas reinhardtii*. Ann Bot 111: 191-205, 2013.
4. Lin CH, Hou RF, Shyu CL, Shia WY, Lin CF and Tu WC: In vitro activity of mastoparan-AF alone and in combination with clinically used antibiotics against multiple-antibiotic-resistant *Escherichia coli* isolates from animals. Peptides 36: 114-120, 2012.
5. Sowa NA, Street SE, Vihko P and Zylka MJ: Prostatic acid phosphatase reduces thermal sensitivity and chronic pain sensitization by depleting phosphatidylinositol 4,5-bisphosphate. J Neurosci 30: 10282-10293, 2010.
6. Hajnoczky G, Davies E and Madesh M: Calcium signaling and apoptosis. Biochem Biophys Res Commun 304: 445-454, 2003.
7. Newmeyer DD and Ferguson-Miller S: Mitochondria: releasing power for life and unleashing the machineries of death. Cell 112: 481-490, 2003.
8. Grześk G, Wiciński M, Malinowski B, Grześk E, Manysiak S, Odrowąż-Sypniewska G, Darvish N and Bierwagen M: Calcium blockers inhibit cyclosporine A-induced hyperreactivity of vascular smooth muscle cells. Mol Med Rep 5: 1469-1474, 2012.
9. Grześk G, Kozinski M, Navarese EP, Krzyzanowski M, Grześk E, Kubica A, Siller-Matula JM, Castriota F and Kubica J: Ticagrelor, but not clopidogrel and prasugrel, prevents ADP-induced vascular smooth muscle cell contraction: a placebo-controlled study in rats. Thromb Res 130: 65-69, 2012.
10. Kanagy NL and Webb RC: Enhanced vascular reactivity to mastoparan, a G protein activator, in genetically hypertensive rats. Hypertension 23: 946-950, 1994.
11. Birnbaumer L: The discovery of signal transduction by G proteins: a personal account and an overview of the initial findings and contributions that led to our present understanding. Biochim Biophys Acta 1768: 756-771, 2007.
12. Cotecchia S: The α_1-adrenergic receptors: diversity of signaling networks and regulation. J Recept Signal Transduct Res 30: 410-419, 2010.
13. Bylund DB, *et al*: Adrenoceptors. In: The IUPHAR Compendium of Receptor Characterization and Classification. 2nd edition. IUPHAR Media, London, pp88-103, 2000.
14. Higashijima T, Burnier J and Ross EM: Regulation of G_i and G_o by mastoparan, related amphiphilic peptides, and hydrophobic amines. Mechanism and structural determinants of activity. J Biol Chem 265: 14176-14186, 1990.
15. Perianin A and Snyderman R: Mastoparan, a wasp venom peptide, indentifies two discrete mechanisms for elevating cytosolic calcium and inositol triphosphates in human polymorphonuclear leukocytes. J Immunol 143: 1669-1673, 1989.
16. Dostal DE, Murahashi T and Peach MJ: Regulation of cytosolic calcium by angiotensins in vascular smooth muscle. Hypertension 15: 815-822, 1990.

17. Argiolas A and Pisano JJ: Facilitation of phospholipase A2 activity by mastoparans, a new class of mast cell degranulating peptides from wasp venom. J Biol Chem 258: 13697-13702, 1983.
18. Wallace MA and Carter HR: Effects of the wasp venom peptide, mastoparan, on a phosphoinositide-specific phospholipase C purified from rabbit brain membranes. Biochim Biophys Acta 1006: 311-316, 1989.
19. Hiramatsu Y, Horn VJ, Baum BJ and Ambudkar IS: Characterization of polyphosphoinositide-specific phospholipase C in rat parotid gland membranes. Arch Biochem Biophys 297: 368-376, 1992.
20. Grześk G and Szadujkis-Szadurski L: Modyfikacja reaktywnosci receptorow α-adrenergicznych przez 8Br-cGMP i angiotensyne II. Ann Acad Med Bydg 17: 5-10, 2003 (In Polish).