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**MODULATORY EFFECT OF SODIUM NITROPRUSSIDE AND 8Br-cGMP ON MASTOPARAN-7 INDUCED CONTRACTION****MODULUJĄCY WPŁYW NITROPRUSYDKU SODU I 8BR-CGMP NA REAKTYWNOŚĆ MIĘŚNIÓWKI INDUKOWANY MASTOPARANEM-7**

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**S u m m a r y**

**Introduction.** Mastoparan-7 activates G-protein and stimulates apoptosis, but in smooth muscle cells leads to increase in perfusion pressure.

Main aim of this study was to evaluate the modulatory effect of 8Br-cGMP and sodium nitroprusside on vascular smooth muscle contraction induced by direct stimulation of G-protein with mastoparan-7.

**Material and methods.** Experiments were performed on isolated and perfused tail artery of Wistar rats. Contraction force in our model was measured by increased level of perfusion pressure with a constant flow.

**Results.** Concentration-response curves obtained for mastoparan-7 presented an sigmoidal relation. In presence of SNP and 8Br-cGMP the significant and dose dependent leftward shift with significant reduction in maximal responses was present. Moreover, analyzing function of calcium stores the significant inhibition of influx from both, intra- and extracellular calcium stores was present.

**Conclusion.** Results of our experiments suggest that contraction induced by direct activation of G-protein with mastoparan-7 may be effectively inhibited in the presence of donors of nitric oxide such as sodium nitroprusside and in the presence of 8Br-cGMP.

**S t r e s z c z e n i e**

Mastoparan-7 jest aktywatorem białka G, stymulującym apoptozę. W mięśniach gładkich naczyń krwionośnych zwiększa kurczliwość.

**Celem** prowadzonego badania było określenie wpływu nitroprusydku sodowego oraz 8Br-cGMP na skurcz mięśni gładkich wywołany bezpośrednią stymulacją białka G przez mastoparan-7.

**Materiał i metody.** Badania przeprowadzono na izolowanych i perfundowanych tętnicach ogonowych szczurów szczepu Wistar. Wykładnikiem skurczu w układzie doświadczalnym były zmiany ciśnienia perfuzyjnego.

**Wyniki.** Krzywe zależności efektu od stężenia agonisty uzyskane dla mastoparanu-7 miały przebieg odpo-

wiadający krzywej sigmoidalnej. W obecności nitroprusydku sodowego i 8Br-cGMP zaobserwowano istotne przesunięcie krzywych w stronę prawą, tj. w stronę wyższych stężeń agonisty z jednoczesną redukcją efektu maksymalnego. Oceniając efektywność skurczu wywołanego napływem wapnia z przestrzeni wewnątrzkomórkowej i zewnątrzkomórkowej, stwierdzono istotne zmniejszenie napływu jonów wapnia z obydwu wymienionych magazynów.

**Wnioski.** Wyniki przeprowadzonych doświadczeń sugerują, że skurcz wywołony przez bezpośrednią aktywację białka G przez mastoparan-7 może być skutecznie hamowany przez donory tlenku azotu, jak np. nitroprusydek sodowy a także w obecności 8Br-cGMP.

**Key words:** mastoparan-7, mastoparan-17, phospholipase C, G-protein, 8Br-cGMP, sodium nitroprusside

**Słowa kluczowe:** mastoparan-7, mastoparan-17, fosfolipaza C, białko G, 8Br-cGMP, nitroprusydek sodowy

## Abbreviations

CRC, concentration response curve  
EC<sub>50</sub>, half maximal effect concentration  
E<sub>max</sub>, maximal tissue response  
mas-7, mastoparan-7  
PLC, phospholipase C

## INTRODUCTION

Mastoparan-7 is a basic tetradecapeptide isolated from wasp venom. The mechanism of its action is related to activation of G protein. Mastoparan-7 catalyze guanosine 5'-diphosphate/guanosine 5'-triphosphate (GDP/GTP) exchange, thus mastoparan-7 acts as activated G protein-coupled receptors. The peptide has been shown to stimulate PLC in several cellular compartments like rat mast cells, rat hepatocytes, and human HL-60 leukaemia cells. In contrast, inhibition of PLC by mastoparan has been demonstrated in SH-SY5Y human neuro-blastoma cells and in human astrocytoma cells [1]. Nitric oxide is most powerful agent released from vascular endothelium responsible for vasorelaxation. 8Br-cGMP is synthetic compound, analog of second messenger of nitric oxide in vascular smooth muscle cells [2, 3, 4].

Calcium ions play the council role in the cell life. Accordingly to pathological factors, Ca<sup>2+</sup> concentration changes occur in the various cell compartments, which may induce apoptosis [5, 6, 7].

Our previous studies confirmed in isolated resistant artery model that mastoparan-7 is able to increase the calcium load in smooth muscle cytoplasm by activation of calcium influx from intra and extracellular calcium stores [8, 9].

Main aim of this study was to evaluate the modulatory effect of 8Br-cGMP and sodium nitroprusside on vascular smooth muscle contraction induced by direct stimulation of G-protein with mastoparan-7.

## METHODS

### Animals

Experiments were performed on isolated and perfused tail artery of Wistar rats (weight 250g to 270 g). Animals were housed under a 12h light/12h dark cycle and had unlimited access to food and water. Rats were narcotized by intraperitoneal injection of 120 mg urethane per 1 kg. Rats were killed by stunning and cervical dislocation. The study protocol was approved

by the Local Ethics Committee. All studies were carried out 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, U.S.A.].

### Drugs and solutions

Mastoparan-7 (G-protein activator), Mastoparan-17 (negative control). Krebs solution contained NaCl (71.8 mM/l), KCl (4.7 mM/l), CaCl<sub>2</sub> (1.7 mM/l) NaHCO<sub>3</sub> (28.4 mM/l), MgSO<sub>4</sub> (2.4 mM/l), KH<sub>2</sub>PO<sub>4</sub> (1.2 mM/l) and glucose (11.1 mM/l). All reagents were purchased from Sigma Aldrich Chemical Co.

### Study design and conduction

After dissection from surrounding tissues, 2-3 cm long segment of a rat tail artery was cannulated and connected to a perfusion device. The distal part was weighted with 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. We gradually increased perfusion solution flow using a peristaltic pump to 1mL/min, until the optimum perfusion pressure 2-4kPa [10].

### Data analysis and statistical procedures

Investigations were performed on TSZ-04 system, Experimetria Ltd. Budapest. Perfusion pressure was measured on BPR-01 and BPR-02 devices, vascular smooth muscle tension was measured on FSG-01 transducer connected with digital recorder Graphtec midi Logger GL820. All transducers used in our experiments were made by Experimetria Ltd, Budapest. Peristaltic pump was made by ZALIMP. Concentration-response curves (CRCs) were calculated according to the van Rossum method. Maximum response of tissue (E<sub>max</sub>) was calculated as a percent of maximal response for phenylephrine. Half maximal effective concentration (EC<sub>50</sub>) was estimated using classical pharmacologic methods with pD2 the negative logarithm of the EC 50. We used the number of the CRC and E<sub>max</sub> in all calculations estimating the statistical significance. As negative control mastoparan-17 has been used.

Results are presented as mean values ± standard deviation. Statistical analysis was performed using the ANOVA test for multiple comparison of means.

Values of  $p$  below 0.05 were considered statistically significant.

## RESULTS

Concentration-response curve obtained for mastoparan-7 presented a sigmoidal relation. In presence of SNP and 8Br-cGMP the significant leftward shift with significant reduction in maximal responses was present. This effect was dose dependent (Figure 1 and 2). For all points for effect 20% or more the differences were statistically significant. Calculated  $E_{max}$ ,  $EC_{50}$  and  $pD_2$  values were presented in Table 1.

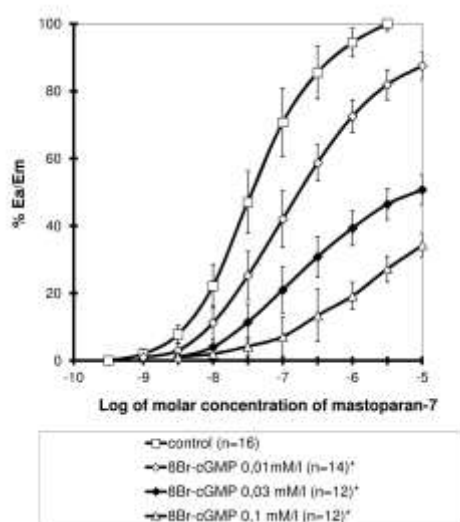


Fig. 1. Concentration response curves for mastoparan-7 in the control and in the presence of sodium nitroprusside

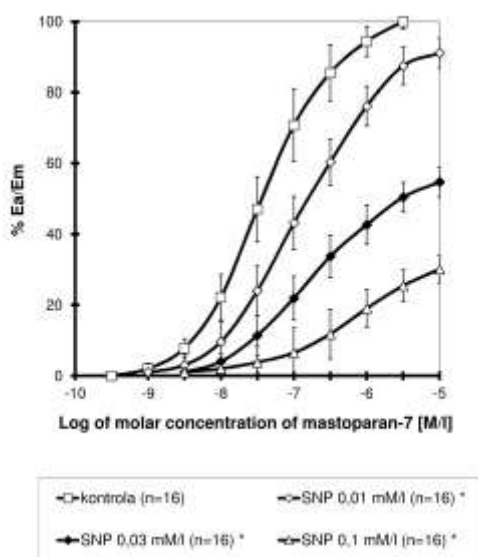


Fig. 2. Concentration response curves for mastoparan-7 in the control and in the presence of 8Br-cGMP

Table 1.  $EC_{50}$ , maximal response and relative potency for mastoparan-7 for controls and in the presence of sodium nitroprusside (PTX) and 8Br-cGMP

	$n^1$	$\%E_{max}^2$	$EC_{50}$ [M/l]	$pD_2$	$RP^3$	$p$
control	16	100	$4.41 (\pm 2.33) \times 10^{-8}$	$7.40 \pm 0.20$	1.000	—
SNP 0.01 mM/l	16	$91 \pm 9$	$2.26 (\pm 0.58) \times 10^{-7}$	$6.66 \pm 0.13$	0.195	<0.0001
SNP 0.03 mM/l	16	$55 \pm 8$	$3.13 (\pm 0.73) \times 10^{-7}$	$6.51 \pm 0.09$	0.141	<0.0001
SNP 0.1 mM/l	16	$30 \pm 6$	$7.51 (\pm 0.32) \times 10^{-7}$	$6.12 \pm 0.20$	0.059	<0.0001
8Br-cGMP 0.01 mM/l	14	$88 \pm 11$	$1.61 (\pm 0.79) \times 10^{-7}$	$6.84 \pm 0.20$	0.274	<0.0001
8Br-cGMP 0.03 mM/l	12	$51 \pm 8$	$2.89 (\pm 0.84) \times 10^{-7}$	$6.56 \pm 0.13$	0.153	<0.0001
8Br-cGMP 0.1 mM/l	12	$34 \pm 8$	$7.15 (\pm 0.82) \times 10^{-7}$	$6.15 \pm 0.05$	0.062	<0.0001

<sup>1</sup> - number of concentration-response curves used for calculations, <sup>2</sup> -  $E_{max}$  - calculated as a percent of maximal response for controls, <sup>3</sup> - RP - relative potency - calculated as  $EC_{50}$  for controls /  $EC_{50}$ , <sup>4</sup> -  $p$  - value calculated in comparison to control values

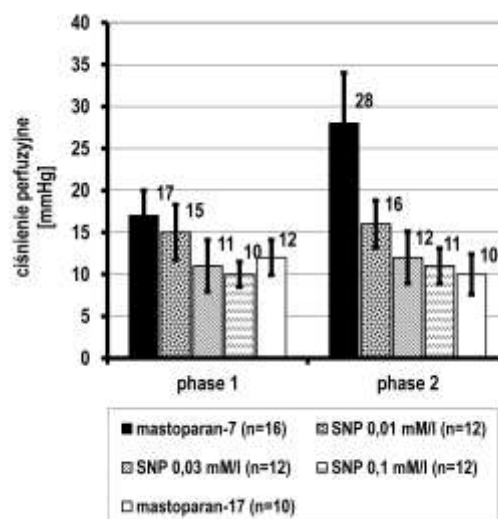


Fig. 3. Impact of G-protein activation by mastoparan 7, on perfusion pressure triggered with intra- and extracellular calcium pool in comparison to the control (mastoparan 17) and in the presence of sodium nitroprusside (SNP)

Analyzing the perfusion pressure as a result of intracellular calcium influx for mastoparan-7 the significant increase was observed in comparison to negative control - mastoparan-17. The same relation was observed after extracellular calcium influx to

cytoplasm. In comparison to phenylephrine and vasopressin all values of perfusion pressure after stimulation of G-protein by mastoparan-7 were significantly lower (Figure 3 and 4).

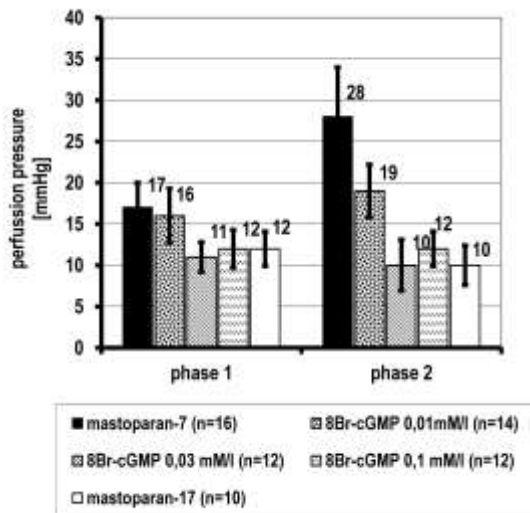


Fig. 4. Impact of G-protein activation by mastoparan 7, on perfusion pressure triggered with intra- and extracellular calcium pool in comparison to the control (mastoparan 17) and in the presence of 8Br-cGMP

## DISCUSSION

In performed experiment, vessel contraction was induced with mastoparan-7, an activator of G-protein. Kanagy et al. [11], investigated spiral cutting fragments of common carotid artery and showed measurable response after 10 minutes, and maximum response after 30 minutes from application of mastoparan-7 ( $10^{-5}$  M/L) [12]. Moreover, in rats with hypertension, arteries reactivity was significantly higher than controls. Nifedipine in the concentration of  $10^{-5}$  M/L, inhibits contraction of VSMC by mastoparan-7 in concentration  $10^{-7}$  M/L. This relationship reveals correlation between voltage-dependent calcium channels and vasoconstriction induced by mastoparan-7. The lack of complete reversal by nifedipine at higher concentrations of mastoparan ( $10^{-5}$  M/L) suggests that an additional mechanism is activated at this higher concentration [7, 11].

In our study impact of donor of nitric oxide – sodium nitroprusside and its second messenger 8Br-cGMP on mastoparan7-induced contraction has been observed. We found a significant inhibition of contraction, triggered by G-protein activation, and

proportional perfusion pressure reduction caused by intra- and extracellular calcium influx in the presence of both modulating agents.

Mastoparan-7 penetrates via biological barriers, and binds to G-protein binding site ligand-receptor. It stimulates G-protein in analogical way as activating receptor. Evaluated in biochemical studies, mastoparan's affinity to individual types of G protein is significantly different. Mastoparan-7 shows higher affinity to  $G_i$  and  $G_o$  proteins, than  $G_s$  protein [13]. Perianin et al. demonstrated, that mastoparan-7 can increase calcium ions concentration in cytoplasm through the mechanisms which are unrelated with  $IP_3$  and DAG production [14].

Affinity to  $G_q$ -protein has not been specified so far, however there were performed functional investigations over the process of activation G-protein with mastoparan-7 in vascular smooth muscle cells of carotid artery in rats. The results showed that mastoparan-7 activates  $G_q$ -proteins in vascular smooth muscle cells, and secondarily to the increase of calcium ions concentration in cytoplasm, vasoconstriction has been observed. Moreover, in rats with genetically determined hypertension, contraction of vascular smooth muscle cells was significantly higher, than control group [11]. Mastoparan-7 may activate phospholipase  $A_2$  in concentration of  $5 \times 10^{-5}$  M/L, leads to degranulation of mast cells [15]. In vascular smooth muscle cells, processes of prostanoids production do not modify contraction triggered by mastoparan-7, it is confirmed by experiments performed in the presence of indomethacin. In these studies the significant impact of indomethacin has not been observed. Mastoparan-7 in the concentration  $10^{-5}$  M/L, may impact on vasoconstriction also by additional mechanisms such as calcium channels modulation and voltage-independent calcium channels [14]. Mastoparan-7 demonstrates also direct action on PLC. In low concentration ( $< 3 \times 10^{-6}$  M/L) PLC activation has been inhibited by mastoparan-7, but in higher concentration ( $> 5 \times 10^{-6}$  M/L) direct activation has been found [16, 17]. In our current and previous studies lower concentration of mastoparan-7 ( $3 \times 10^{-10} - 3 \times 10^{-6}$  M/L) has been used [8, 9]. In view of this fact, used concentrations were not high enough to change other, than G-protein elements of signaling pathway. Vasoconstriction induced by mastoparan-7, depends on intra- and extracellular calcium pool, which also may affect apoptosis. The effect of this process was higher values of perfusion pressure.

The constriction of VSMC obtained by mastoparan-7 was significantly lower in comparison to phenylephrine and vasopressin. It was confirmed by Kanagy et al. [11]. The effects observed for mastoparan-7 was similar to the effect occurred after activation by partial agonist, in distinction to full agonist such as phenylephrine or vasopressin. Related perfusion pressure values induced by clonidine –  $\alpha_2$ -receptor agonist moreover inhibitory effect of SNP and 8Br-cGMP was similar to observed during stimulation of G-protein coupled receptors [4].

## CONCLUSIONS

Results of our experiments suggest that contraction induced by direct activation of G-protein with mastoparan-7 may be effectively inhibited in the presence of donors of nitric oxide such as sodium nitroprusside and in the presence of 8Br-cGMP.

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