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Effect of acetylcholine on vascular smooth muscle contraction induced by phenylephrine, angiotensin II and mastoparan-7

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ABSTRACT

Background. The reactivity of blood vessels depends on their structure and the presence of calcium ions. It is modulated by numerous factors which activate specific signalling pathways leading to contraction or relaxation of smooth muscle. The action of various vasodilatation substances may be altered under the influence of similar or quite different modulators.

Main aim. of this study was to assess the role of acetylcholine and calcium ions in modulating the contraction induced by angiotensin II (ANG II), phenylephrine (PHE) and mastoparan-7, a direct activator of G-protein.

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

Results. ANG II caused an increase in perfusion pressure in physiological salt solution (PSS) and calcium free PSS (FPSS).Under the influence of increasing concentrations of acetylcholine, a statistically significant reduction in perfusion pressure in both types of fluid was noted. In the presence of nitro-L-arginine (nitric oxide synthase inhibitor, L-NNA) in both solutions, no changes in contraction stimulated by ANG II or spasmolytic effect of acetylcholine were observed. PHE, in a similar manner to ANG II, caused contraction in FPSS and PSS, which was similarly modulated by acetylcholine. In the mastoparan-7 induced contraction, the pattern of tissue response was similar. For all groups, maximal perfusion pressure in PSS was higher than for FPSS.

Conclusions. The results of our experiments suggest that acetylcholine by activation of vascular endothelium is able to induce dose-dependent vasodilatation not only in contraction related to typical metabotropic receptor agonists, but also after direct stimulation of G-protein with mastoparan-7. This effect may be reversed in the presence of inhibitors of endothelial synthase of nitric oxide.

Key words: calcium ions, adrenoceptors, angiotensin II, mastoparan-7, acetylocholine

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Introduction

The reactivity of blood vessels depends on their structure and the presence of calcium ions [1]. It is modulated by numerous factors which activate specific signalling pathways leading to contraction or relaxation of smooth muscle. The action of various vasodilatation substances may be altered under the influence of similar or quite different modulators. Vascular reactivity may vary with age, which probably is associated with endothelial dysfunction, leading to a reduction in nitric oxide synthesis [2–4]. Angiotensin II (ANG II) triggers vasoconstriction via a metabotropic AT1 receptor [5]. ANG II also regulates smooth muscle cell (SMC) growth, has an effect on apoptosis and migration and has proinflammatory action. In addition, it causes the production of other growth- and contraction-stimulating factors. It is, therefore, important both for maintaining the proper

structure and function of blood vessels and may mediate pathophysiological processes leading to the development of cardiovascular diseases [6]. Phenylephrine (PHE) is an agonist of the α1-adrenergic metabotropic receptor through which it induces vasoconstriction [7].

Both substances, ANG II and PHE, act through G proteins, which leads to stimulation of phospholipase C and the synthesis of secondary messengers: IP3 and DAG [8–10]. IP3 binds to the endoplasmic reticulum membrane (ER) IP3R receptors and causes the release of Ca^{2+} from intracellular pools. The ryanodin receptors (RyR), stimulated, among others, by caffeine [1, 11, 12], are an alternative way for the release of calcium from the ER. Contraction of vascular smooth muscle may also occur via calcium ions escaping from the extracellular space through channels in the cell membrane [receptor-operated Ca^{2+} channels (ROC)] activated by ligands ANG II or PHE [13]. Mastoparan-7 is a basic tetradecapeptide isolated from wasp venom which activates G proteins by catalysing guanosine 5'-diphosphate/guanosine 5'-triphosphate (GDP/GTP) exchange, thus the compound mimics the action of activated G protein-coupled receptors. The peptide has been shown to stimulate PLC in several cellular compartments such as 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 [14–16].

Studies on vas deferens (human and rat) and rat tail arteries have shown that receptor associated G-protein modulation may be influenced by sodium nitroprusside and 8Br-cGMP [17–19]. Nitric oxide derived from endothelium is a major vasodilatation factor [20]. Acetylcholine can stimulate the release of nitric oxide in a cGMP-mediated relaxing effect [21, 22]. However, studies using isolated human placental villous arteries found that NO donors and 8Br-cGMP did not cause relaxation of arteries contracted with caffeine. The mechanism of nitric oxide action on the cardiac calcium release channel in canines has been explored [23, 24]. Lim et al. discussed various ways in which nitric oxide can modulate cardiac ryanodine receptor function and suggested the possibility of pharmacological strategies in heart failure, related to the considered mechanisms [25]. Grześk et al. [26] suggest that calcium movements may be one of most important mechanisms of cyclosporine-A induced hypertension.

The aim of this study was to assess the role of acetylcholine and calcium ions in modulating the contraction induced by ANG II, PHE and mastoparan-7.

Materials and methods

The study was performed on perfunded tail arteries of male Wistar rats weighing 250–350 g, euthanised with an intraperitoneal injection of urethane at the dose of 120 mg/kg. The cannula was introduced in the proximal section of the rat tail artery (2.5–3 cm in length) and combined with a perfusion system and a set that allows constant measurement and recording of perfusion pressure. After loading the distal end of the isolated artery with a weight of 500 mg, the preparation was placed upright in a thermostated vessel for isolated organs 20 mL in volume and oxygenated with physiological fluid at a temperature of 37°C. Perfusion fluid flow was increased gradually to 1 ml/min. Investigations were performed on TSZ-04 system, Experimetria Ltd. Budapest, Hungary. Perfusion pressure was measured on BPR-01 and BPR-02 devices, vascular smooth muscle tension was measured on a FSG-01 transducer connected with digital recorder Graphtec midi Logger GL820. All transducers used in our experiments were made by Experimetria Ltd. Peristaltic pump was made by ZALIMP.

The experiments were carried out to determine the importance of intracellular and extracellular pools of Ca^{2+} in reactions induced by ANG II, PHE, mastoparan-7, and Bay K8644 in control conditions and after addition of L-NNA (nitro-L-arginine — nitric oxide synthase inhibitor) and in the presence of increasing concentrations of acetylcholine using two types of Krebs fluid:

— FPSS — Ca^{2+}-free EGTA-Krebs with the following composition: NaCl (71.8 mM/L), KCl (4.7 mM/L), $NaHCO_3$ (28.4 mM/L), $MgSO_4$ (2.4 mM/L), KH_2PO_4 (1.2 mM/L), glucose (11.1 mM/L) with the addition of EGTA (30 μM/L);

— PSS — fluid with Ca^{2+} EGTA-Krebs (normal) with the following composition: 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), glucose (11.1 mM/L) with the addition of EGTA (30 μM/L), after emptying the intracellular pool of calcium ions.

The increase in pressure of the perfusate in the experimental system was an exponent of vessel spasm. 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.].

Results

ANG II caused an increase in perfusion pressure in FPSS and PSS. Under the influence of increasing concentrations of acetylcholine, a statistically significant reduction in perfusion pressure in both types of fluid was noted. In the presence of L-NNA in both solutions, no changes in contraction stimulated by ANG II or

Table 1. Maximal perfusion pressure during ANGiotensin II, PHEnylephrine and mastoparan-7 induced contraction in the presence of acetylcholine for controls and after inhibition of nitric oxide synthase

			Perfusion pressure [mm Hg]				
Agonist	**n**	**Solution**	**Control**	**Ach 10 nM/L**	**Ach 30 nM/L**	**Ach 0.1 μM/L**	**Ach 0.3 μM/L**
ANG II	14	FPSS	98 ± 12	76 ± 14*	59 ± 12*	38 ± 8*	22 ± 6*
ANG II + L-NNA	15		99 ± 13	96 ± 10	102 ± 10	98 ± 10	96 ± 9
ANG II	14	PSS	125 ± 12	98 ± 12*	84 ± 11*	65 ± 12*	48 ± 10*
ANG II + L-NNA	12		122 ± 10	120 ± 11	155 ± 14	118 ± 12	120 ± 10
PHE	14	FPSS	105 ± 12	81 ± 12*	69 ± 12*	42 ± 11*	25 ± 8*
PHE + L-NNA	12		102 ± 10	101 ± 12	105 ± 14	102 ± 14	98 ± 12
PHE	16	PSS	119 ± 12	97 ± 12*	87 ± 14*	58 ± 11*	50 ± 9*
PHE + L-NNA	13		117 ± 14	118 ± 13	116 ± 14	121 ± 13	122 ± 14
Mastoparan-7	16	FPSS	55 ± 10	36 ± 7*	24 ± 6*	16 ± 2*	15 ± 3*
Mastoparan-7+L-NNA	15		53 ± 10	59 ± 9	55 ± 7	49 ± 10	50 ± 10
Mastoparan-7	16	PSS	60 ± 7	47 ± 8*	36 ± 6*	25 ± 3*	13 ± 4*
Mastoparan-7+L-NNA	14		57 ± 8	60 ± 7	71 ± 7	62 ± 8	60 ± 7

* $P < 0.05$

n — number of CRCs used in calculations; PSS — physiological salt solution; FPSS — calcium free PSS; L-NNA — nitro-L-arginine, nitric oxide synthase inhibitor; ANG — angiotensin; PHE — phenylphrine

spasmolytic effect of acetylcholine were observed. PHE, in a similar manner to ANG II, caused contraction in FPSS and PSS, which was similarly modulated by acetylcholine. In the mastoparan-7 induced contraction, the pattern of tissue response was similar. The difference was in the time from stimulation to maximal response (in PHE or ANG II groups it was several seconds, whereas for mastoparan-7 it was up to 30 minutes). Moreover maximal perfusion pressure in the mastoparan-7 group was lower than for PHE or ANG II. In the presence of acetylcholine, a significant and dose-dependent reduction in perfusion pressure was found. For all groups maximal perfusion pressures in PSS were higher than for FPSS (Tab. 1).

Discussion

Calcium ions are an essential element in muscle contraction. Vascular tone can be adjusted by a variety of substances that stimulate the release of calcium from cellular stores, that cause the influx of calcium from the outside, and that stimulate sensitivity to calcium ions. A key role in regulating muscle function is maintaining the concentration of calcium ions within a very narrow range and regulating their ability for rapid increase [1]. An increase in intracellular calcium levels precedes and induces the contraction of smooth muscle. Acetylocholine decreases arterial tension due to the release of NO from endothelial cells, thus it stimulates the NO/cGMP signalling pathway [27]. Cyclic nucleotides, e.g. cAMP and cGMP, which regulate the function of ion channels and calcium levels in the cell through the appropriate protein kinases, exhibit functional antagonism of calcium ions in smooth muscle [28, 29]. The aim of the present study was to determine the role of acetylcholine and calcium ions in modulating the vascular contraction induced by angiotensin II, phenylephrine and mastoparan-7.

The experiments were carried out in PSS (after emptying the intracellular pool of calcium ions) and FPSS to determine the importance of the extracellular and the intracellular pools of calcium ions. Metabotropic receptor agonists, ANG II and PHE, led to an increase in perfusion pressure in both types of solutions. A similar effect is present after direct stimulation of G-protein with mastoparan-7. The effect of mastoparan-7 seems to be comparable with partial agonists rather than with full agonists such as phenylephrine or angiotensin II [15, 16]. Similar observations were also derived from experiments on rat aorta and human mesenteric arteries [28, 29].

Acetylcholine reduced the vascular contraction stimulated by ANG II and PHE in a concentration-dependent manner. Additionally, in experiments using human mesenteric arteries, such an effect of acetylcholine was observed [22]. Ji et al. in studies of rat aorta demonstrated that the inhibitory effect of acetylcholine was associated with the presence of endothelial cells, and this effect was not present in experiments carried out in arteries denuded of endothelium [30]. Another series of studies found that blocking NO synthase (after the addition of L-NNA) led to the elimination of the relaxing effects of

acetylcholine. These results confirm the dependence of acetylcholine on NO synthesis.

In the present study, the importance of calcium ions and acetylcholine, as an element in the Ach/NO/cGMP signalling pathway in the vascular contraction induced by ANG II and PHE through metabotropic receptors (AT1 and α1-adrenergic, respectively) was compared with the action of mastoparan-7, a direct activator of G-protein. The action of ANG II and PHE was mediated by two pools of calcium. Direct activation of G-protein with mastoparan-7 activates both calcium pools too. The relaxing effect of acetylcholine on responses stimulated by ANG II, PHE and mastoparan-7 indicates the participation of endothelium derived nitric oxide in modulating the reactivity of arteries to the studied agonists.

Conclusion

The results of our experiments suggest that acetylcholine by activation of vascular endothelium is able to induce dose-dependent vasodilatation not only in contraction related to typical metabotropic receptor agonists, but also after direct stimulation of G-protein with mastoparan-7. This effect may be reversed in the presence of inhibitors of endothelial synthase of nitric oxide.

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