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EFFECT OF THE VASCULAR ENDOTHELIUM ON CONTRACTIONS INDUCED BY NORADRENALINE AND PHENYLEPHRINE IN PERFORATING BRANCH OF THE HUMAN INTERNAL MAMMARY ARTERY

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Effect of the vascular endothelium on contractions induced by noradrenaline and phenylephrine in perforating branch of the human internal mammary artery. S. PEŠIĆ, L. GRBOVIĆ, A. JOVANOVIĆ, M. RADENKOVIĆ, D. STOJIĆ. Pol. J. Pharmacol., 2003, 55, 581–593.

The effects of noradrenaline (Nor) and phenylephrine (Phe) on the isolated, non-precontracted perforating branch of the human internal mammary artery (HIMA) were investigated. Nor and Phe induced concentration-dependent contractions of intact and endothelium-denuded arterial rings with no statistically significant differences between the pEC_{50} and maximal response values. The pretreatment of arterial rings with indomethacin had no effect on Nor- and Phe-induced contractions of both, intact and endothelium-denuded preparations. The pre-addition of L-NMMA did not affect contractions of perforating branch of the HIMA evoked by Nor, but provoked significant potentiation of Phe-induced contractions of perforating branch of the HIMA both intact and denuded of endothelium only at Phe concentration higher than 3×10^{-6} M. The effects of selective α_1-adrenoceptor antagonist, prazosin and selective α_2-adrenoceptor antagonist, rauwolscine were concentration-dependent, and they induced a significant shift to the right (for both studied antagonists) of the concentration-response curves for Nor in both preparations with or without endothelium. The effects of prazosin and rauwolscine on the concentration-response curves for Phe were similar. In conclusion, this study has shown that Nor and Phe induce concentration-dependent contractions of the perforating branch of the HIMA. Removal of the endothelium did not modify this effect. Products of cyclooxygenase pathway had no influence on Nor and Phe action. Endothelium derived nitric oxide (NO) had no modulatory effect of Nor-induced contractions, but inhibition of NO synthesis provoked potentiation of Phe-induced contractions either in intact or endothelium-denuded preparations. The mechanism of this effect remains still unclear. On the basis of differential affinity of the antagonists and affinities of Nor and Phe themselves, we suggest that α_1-adrenoceptor subtype is probably involved in the Nor- and Phe-induced contraction of the perforating branch of the HIMA both intact or denuded of endothelium.

Key words: *vasoconstriction, noradrenaline, phenylephrine, endothelium, adrenoceptors, perforating branch of HIMA*

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INTRODUCTION

It has been known for some time that removal of vascular endothelium can potentiate the responses of some animal arteries to exogenously applied vasoconstrictor substances, especially to noradrenaline [8, 25]. This effect was also demonstrated in a main tree of the human internal mammary artery (HIMA) [31]. Moreover, Alosachie and Godfraind [2] showed that in the certain blood vessels, removal of endothelium enhanced receptor reserve for noradrenaline. Contrary, results of some recent investigation showed that contraction of some animal and human arteries, induced by noradrenaline, was unaffected by removal of endothelium or pretreatment with nitric oxide (NO) synthase inhibitors [4, 18, 33]. At the same time, there are relatively few facts related to the possible modulatory effect of vascular endothelium to constrictive action of phenylephrine.

In humans, perforating branch of internal mammary artery supplies a major part of mammary gland tissue [30]. However, the effect of the vascular endothelium on noradrenaline- and phenylephrine-mediated responses and the precise receptor mechanism of their constrictive action in the perforating branch of HIMA has not been studied yet.

Therefore, the purposes of this study were to examine and to compare the influence of the vascular endothelium on noradrenaline (combined α- and β-adrenoceptor stimulator)- and phenylephrine (specific α_1-stimulator)-induced contractions of the perforating branch of HIMA and to determine the adrenergic receptor type mediating this action.

MATERIALS and METHODS

Perforating branches of HIMA were obtained from 67 women (mean age ± SEM 54.8 ± 1.2, range 30–63 years) undergoing mammectomy. No patients receiving radiological, cytotoxic or antihypertensive therapy were included. We chose the perforating branch of HIMA as the type of artery with outer diameter of 1–2 mm. Arteries from patients undergoing mammectomy for breast cancer, without chemotherapy were used. Therefore, we consider that human arteries that we used in this study represent the normal conditions of endothelium-derived relaxing factors similarly to Urakami-Harasawa et al. [37], who used gastroepiploic arteries from the patients who underwent gastrectomy for gastric cancer. During operation, the patients received anesthesia with a combination of nitrous oxide, oxygen, thiopentone and fentanyl. Muscle relaxation was induced by suxamethonium and maintained by pancuronium. The vessel was excised within 10 min of clamping at place where it perforates pectoral muscle and placed in cold (4°C) Krebs-Ringer-bicarbonate solution. The patients were informed in detail about the purpose of the investigation and had given their informed consent. After excision, the vessels were immediately transported to the laboratory.

Vascular preparations

The perforating branches of HIMA (outer diameter 1–2 mm) were dissected free from connective tissue. They were cut into 3-mm rings. Care was taken not to damage the endothelium. In some rings, the endothelium was removed mechanically by gentle rubbing of the intimal surface with stainless-steel wire [20]. Ring preparations were mounted between two stainless steel triangles in an organ chamber containing 15 ml of Krebs-Ringer-bicarbonate solution (37°C, pH 7.4) aerated with 95% O_2 and 5% CO_2. One of the triangles was attached to a displacement unit allowing the fine adjustment of tension and the other was connected to a force-displacement transducer (Hugo Sachs K30). Isometric tension was recorded on a model Hugo Sachs MC 6621 recorder and expressed in mN. Preparations were allowed to equilibrate for 60 min in Krebs-Ringer-bicarbonate solution. During this period the organ baths were washed with fresh (37°C) buffer solution every 15 min. Each ring was gradually stretched to the optimal point of its length-tension curve (19 ± 1 mN, range 15–23 mN) as determined by the tension developed in response to potassium chloride (4×10^{-2} mol/l) added at each stretch level. After optimal length was obtained, the segments were allowed to equilibrate for 30 min before experimentation.

Experimental procedure

At the beginning and the end of each experiment, the contractile response to 100 mmol/l KCl solution was obtained. Only if the response to KCl was similar in magnitude (with variation less than 10%), the data from this particular experiment were included in this analysis.

Concentration-response curves for noradrenaline and phenylephrine were constructed by adding

increasing concentrations of agonist when the previous concentration had produced its equilibrium response, or 5 min if no response was obtained.

Prior to the beginning of experiment, in order to confirm the presence or successful denudation of endothelium, the rings were precontracted with 100 mmol/l of KCl and challenged with acetylcholine (ACh) (10^{-5} mol/l). On the basis of the prior study [19, 28], relaxation greater than 50% of the maximal relaxation evoked by ACh was indicative of structurally intact endothelium (maximal relaxation represented complete return to the resting tension from the contraction in response to KCl) [19, 20]. Additionally, at the end of some experiments (10 of each), the condition of the endothelium was verified by Van Gieson's staining with iron hematoxylin and light microscopic examination of the intimal surface [11]. Concentration-response curves were obtained by cumulative addition of noradrenaline and phenylephrine ($10^{-10} – 3 \times 10^{-4}$mol/l) to non-precontracted ring segments. Experiments were prepared using a multiple curve design. Therefore, the following protocol was used: (i) contraction in response to KCl, followed by addition of ACh, three washes and a 30 min equilibration period; (ii) concentration-response curve for noradrenaline or phenylephrine (used as the tissue control), followed by three washes, addition of the antagonists, 30 and 40 min equilibration period for N^G-monomethyl-L-arginine acetate (L-NMMA) and indomethacin, respectively, and 60 min equilibration period for receptor antagonists; (iii) second concentration-response curve for noradrenaline or phenylephrine, followed by three washes and a 30 min equilibration period; (iv) contraction in response to KCl and addition of ACh. When prazosin and rauwolscine were used, three different concentrations of antagonist were used on the same day, but with only one concentration of antagonist per ring. All experiments were performed on both preparations intact and denuded of endothelium.

At the beginning of our investigation, in a separate series of experiments, concentration-response curves for noradrenaline were made in the presence of 10^{-5} mol/l hydrocortisone, 10^{-5} mol/l desipramine, and 10^{-5} mol/l propranolol to block neuronal and extraneuronal uptake of noradrenaline and β-adrenoceptors, respectively [13]. Since these compounds did not affect the concentration-response curves for noradrenaline in the studied vessels, hydrocortisone, desipramine, and propranolol were excluded from further study.

Data processing and statistics

The contraction induced by each concentrations of noradrenaline or phenylephrine was expressed as a percentage of the maximum contraction to noradrenaline or phenylephrine (3×10^{-4} mol/l). The concentration of noradrenaline or phenylephrine eliciting 50% of its own maximum response (EC_{50}) was determined for each curve by linear interpolation. EC_{50} value was presented as pEC_{50} ($pD_2 = –\log EC_{50}$).

For the assessment of the effect of the α-adrenoceptor antagonists, three different concentrations of the antagonist were examined. The pA_2 values (–log molar concentration of antagonist reducing the agonist EC_{50} by factor of two) for prazosin and rauwolscine were determined from Schild plots [3] using noradrenaline or phenylephrine as the agonist. The concentration ratios (the ratio between the EC_{50} value for noradrenaline or phenylephrine in the presence and absence of an antagonist) at different antagonist concentrations for the different noradrenaline or phenylephrine/antagonist pairs were plotted in a Schild diagram using regression analysis, and pA_2 was obtained from the intercept of the regression line with the abscissa [3]. The significance of the Schild plot linearity was tested by analysis of variance [21]. The closeness of the slope to unity was verified by *t*-test and was considered not different from unity if $p > 0.05$.

The results are expressed as means ± SEM or as mean values with 95% confidence intervals; *n* refers to the number of women from whom vessels were taken. Statistical differences between two means were evaluated with Student's *t*-test for paired or unpaired observations where appropriate. A value of $p < 0.05$ was considered to be statistically significant. The least squares method was used for calculating linear regressions.

Drugs and solutions

The Krebs-Ringer-bicarbonate solution had the following composition (in mmol/l): NaCl 118.3; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25.0; EDTA 0.026; glucose 11.1. The solution was continuously bubbled with 95% O_2 and 5% CO_2 resulting in pH 7.4 and temperature was kept at 37°C. The following drugs were used: noradrenaline bitartrate, L-phenylephrine hydrochloride, ACh chlo-

ride, prazosin hydrochloride, indomethacin, propranolol, desipramine, hydrocortisone (Sigma, USA), rauwolscine hydrochloride (RBI, USA), N^G-monomethyl-L-arginine acetate (L-NMMA) (Wellcome, UK). All drugs were prepared immediately before the experiment and stored on ice until use. Indomethacin was dissolved in equimolar Na_2CO_3 solution All other agents were dissolved in distilled water and diluted to the desired concentration with buffer. All drugs were added directly to the bath in a volume of 150 µl and the given concentrations are the calculated final concentration in the bath solution.

RESULTS

Effect of noradrenaline and phenylephrine on the perforating branch of the HIMA

Noradrenaline (10^{-10} – 3×10^{-4} mol/l) induced concentration-dependent contraction of intact and endothelium-denuded arterial rings (intact: pEC_{50} = 6.48 ± 0.02, maximal response = 29.87 ± 3.5 mN; denuded: pEC_{50} = 6.61 ± 0.006, maximal response = 29.16 ± 4.2 mN, n = 30). The removal of endothelium slightly enhanced contractions of arterial rings of perforating branch of the HIMA to noradrenaline, but the difference between the pEC_{50} and maximal response values was not statistically significant ($p > 0.05$) (Fig. 1A).

The presence of hydrocortisone, desipramine and propranolol in the bath solution during experiments did not affect the concentration-response curve for noradrenaline in perforating branch of the HIMA either intact or denuded of endothelium (data not shown).

Phenylephrine (10^{-10} – 3×10^{-4} mol/l) induced concentration-dependent contraction of intact and endothelium-denuded arterial rings of perforating branch of the HIMA (intact: pEC_{50} = 6.80 ± 0.02, maximal response = 32.10 ± 4.1 mN; denuded: pEC_{50} = 6.74 ± 0.03, maximal response = 31.15 ± 3.9 mN, n = 37) and there was not statistically significant difference between the pEC_{50} and maximal response values ($p > 0.05$) (Fig. 1B).

Effect of indomethacin and L-NMMA on noradrenaline- and phenylephrine-induced contractions of the perforating branch of the HIMA

The pretreatment of arterial rings with indomethacin (10^{-5} mol/l) had no effect on noradrenaline-induced contractions of both, intact (pEC_{50}: 6.17 ± 0.02 vs. 6.36 ± 0.04, maximal response: 31.14 ± 4.1 mN vs. 29.94 ± 3.5 mN, n = 3) and endothelium-denuded preparations (pEC_{50}: 6.35 ± 0.05 vs. 6.40 ± 0.04, maximal response: 29.19 ± 3.8 mN vs. 28.82 ± 3.4 mN, n = 3) (Fig. 2A, B).

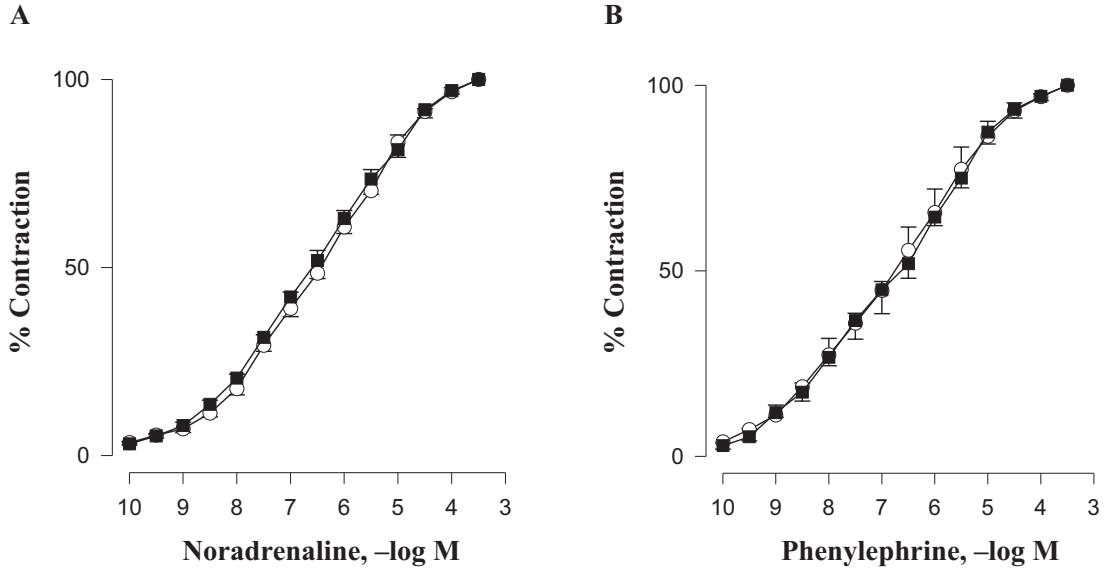

Fig. 1. Concentration-response curves for noradrenaline (A) and phenylephrine (B) in perforating branch of the HIMA with intact (○) and denuded (■) endothelium. Each point represents mean ± SEM (n = 30 for noradrenaline, n = 37 for phenylephrine). Responses are expressed as a percentage of the maximum contraction evoked by agonist itself (3×10^{-4} mol/l)

Indomethacin (10^{-5} mol/l) exerted similar effects on phenylephrine-induced contractions of perforating branch of the HIMA (intact endothelium pEC_{50}: 6.76 ± 0.03 vs. 6.71 ± 0.02, maximal response: 30.09 ± 4.4 mN vs. 30.25 ± 2.4 mN, n = 3; endothelium-denuded pEC_{50}: 6.35 ± 0.05 vs. 6.40 ± 0.04, maximal response: 29.19 ± 3.8 mN vs. 28.82 ± 3.4 mN, n = 3) (Fig. 2C, D).

The pre-addition of L-NMMA (10^{-5} mol/l) did not affect contractions of perforating branch of the HIMA evoked by noradrenaline (intact endothelium pEC_{50}: 6.43 ± 0.04 vs. 6.50 ± 0.06, maximal response: 31.06 ± 3.1 mN vs. 30.04 ± 2.2 mN, n = 4; endothelium denuded pEC_{50}: 6.74 ± 0.06 vs. 6.54 ± 0.02, maximal response: 28.34 ± 4.5 mN vs. 27.98 ± 4.8 mN, n = 4) (Fig. 3A, B).

Contrary, L-NMMA provoked significant potentiation of phenylephrine-induced contractions of perforating branch of the HIMA with intact endothelium (pEC_{50}: 6.15 ± 0.02 vs. 6.01 ± 0.07, maximal response: 59.88 ± 7.4 mN vs. 31.52 ± 3.1 mN, n = 6). L-NMMA potentiated also the response of arterial rings denuded of endothelium to phenylephrine, but only at its concentration higher than

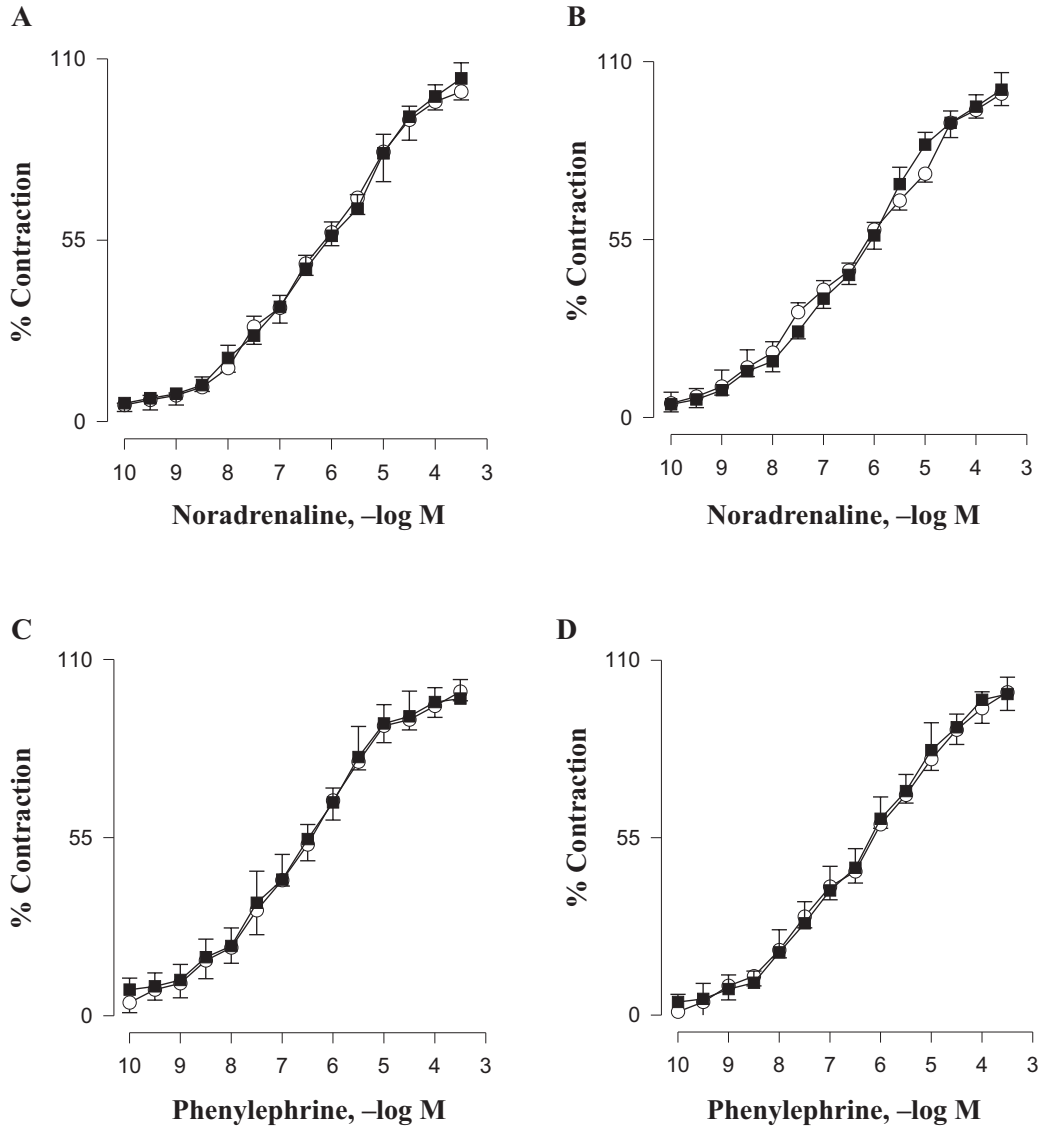

Fig. 2. Concentration-response curves for noradrenaline and for phenylephrine in perforating branch of the HIMA with intact and denuded endothelium (A, B for noradrenaline and C, D for phenylephrine, respectively) in the absence (○) and presence (■) of indomethacin. Each point represents mean ± SEM (n = 3 for noradrenaline, n = 4 for phenylephrine). Responses are expressed as a percentage of the maximum contraction evoked by agonist itself (3×10^{-4} mol/l)

3×10^{-6} M (pEC_{50}: 6.50 ± 0.3 vs. 6.67 ± 0.09, maximal response: 45.88 ± 6.6 mN vs. 31.44 ± 4.2 mN, n = 6) (Fig. 3C, D).

Effect of prazosin and rauwolscine on noradrenaline- and phenylephrine-induced contractions of the perforating branch of the HIMA

The effects of selective α_1-adrenoceptor antagonist, prazosin ($4 \times 10^{-10} – 4 \times 10^{-9}$ mol/l, n = 4–12) and selective α_2-adrenoceptor antagonist rauwolscine ($10^{-6} – 10^{-5}$ mol/l, n = 4–12) were concentration-dependent and induced a significant shift to the right ($p < 0.01$ for both studied antagonists) of the concentration-response curves for noradrenaline on both preparations with or without endothelium (Fig. 4A–D). The data from the Schild plots with adrenoceptor antagonists were analyzed as described by Arunlakshana and Schild [3]. The experiments with prazosin and rauwolscine yielded straight lines with mean slopes not different from unity (intact endothelium: prazosin: 0.913 ± 0.03, rauwolscine: 0.96 ± 0.1; denuded of endothelium:

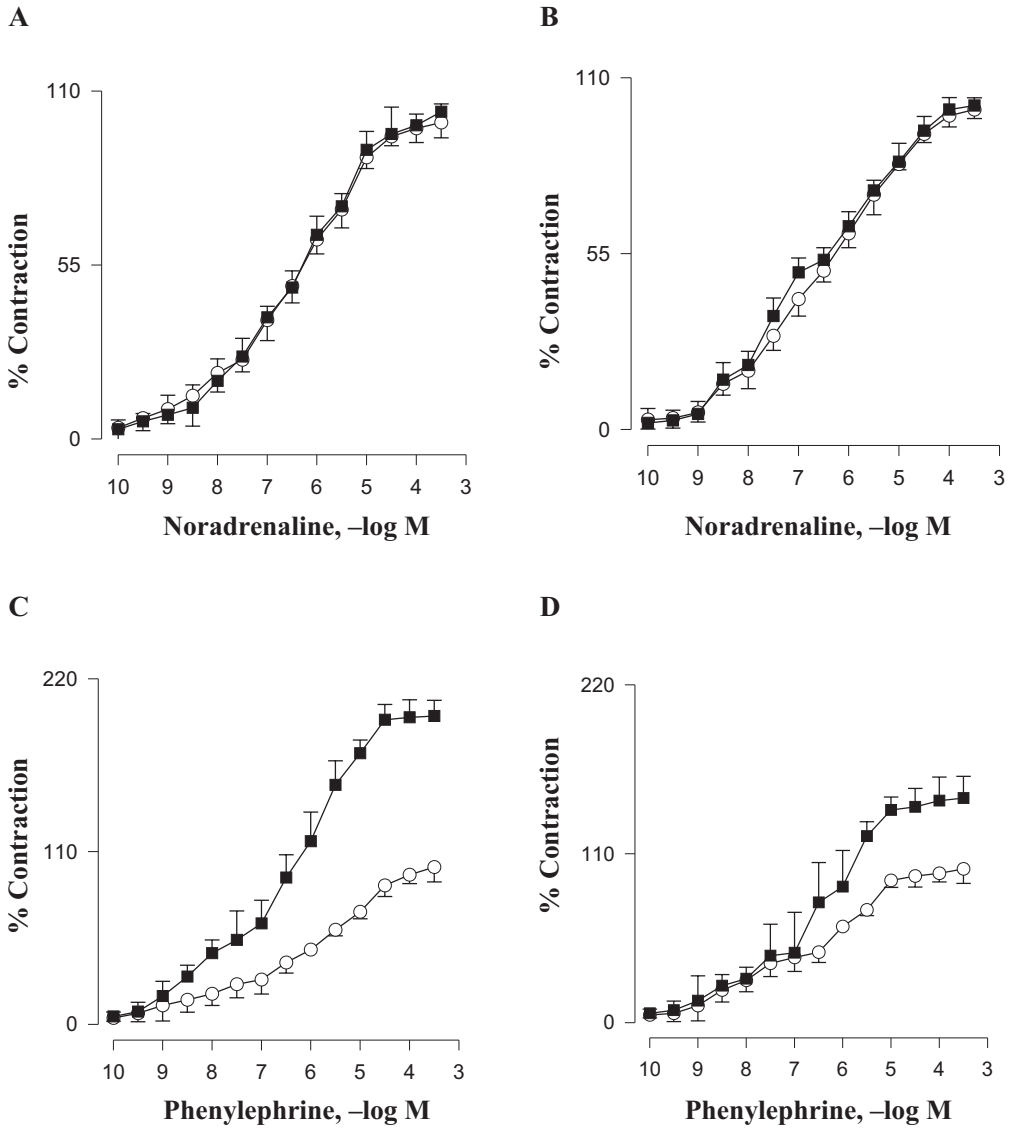

Fig. 3. Concentration-response curves for noradrenaline and for phenylephrine in perforating branch of the HIMA with intact and denuded endothelium (A, B for noradrenaline and C, D for phenylephrine, respectively) in the absence (○) and presence (■) of L-NMMA. Each point represents mean ± SEM (n = 3 for noradrenaline, n = 6 for phenylephrine). Responses are expressed as a percentage of the maximum contraction evoked by agonist itself (3×10^{-4} mol/l)

prazosin: 1.065 ± 0.19, rauwolscine: 0.91 ± 0.09). The intercept of the line with the abscissa was 9.56 ± 0.03 and 9.60 ± 0.15 for prazosin and 6.11 ± 0.08 and 6.31 ± 0.08 for rauwolscine effect on arterial rings intact and endothelium-denuded, respectively (Tab. 1, Fig. 6A).

The effects of selective α_1-adrenoceptor antagonist, prazosin ($4 \times 10^{-10} – 4 \times 10^{-9}$ mol/l, n = 5–15) and selective α_2-adrenoceptor antagonist rauwolscine ($10^{-6} – 10^{-5}$ mol/l, n = 4–12) were concentration-dependent and induced a significant shift to the right ($p < 0.01$ for both studied antagonists) of the concentration-response curves for phenylephrine effect on both preparations with or without endothelium (Fig. 5A–D). The data from the Schild plots with adrenoceptor antagonists were analyzed as described by Arunlakshana and Schild [3]. The experiments with prazosin and rauwolscine yielded straight lines with mean slopes not different from unity (intact endothelium: prazosin: 1.09 ± 0.06, rauwolscine: 1.04 ± 0.04; denuded of endothelium: prazosin: 1.1 ± 0.1, rauwolscine: 0.96 ± 0.13). The

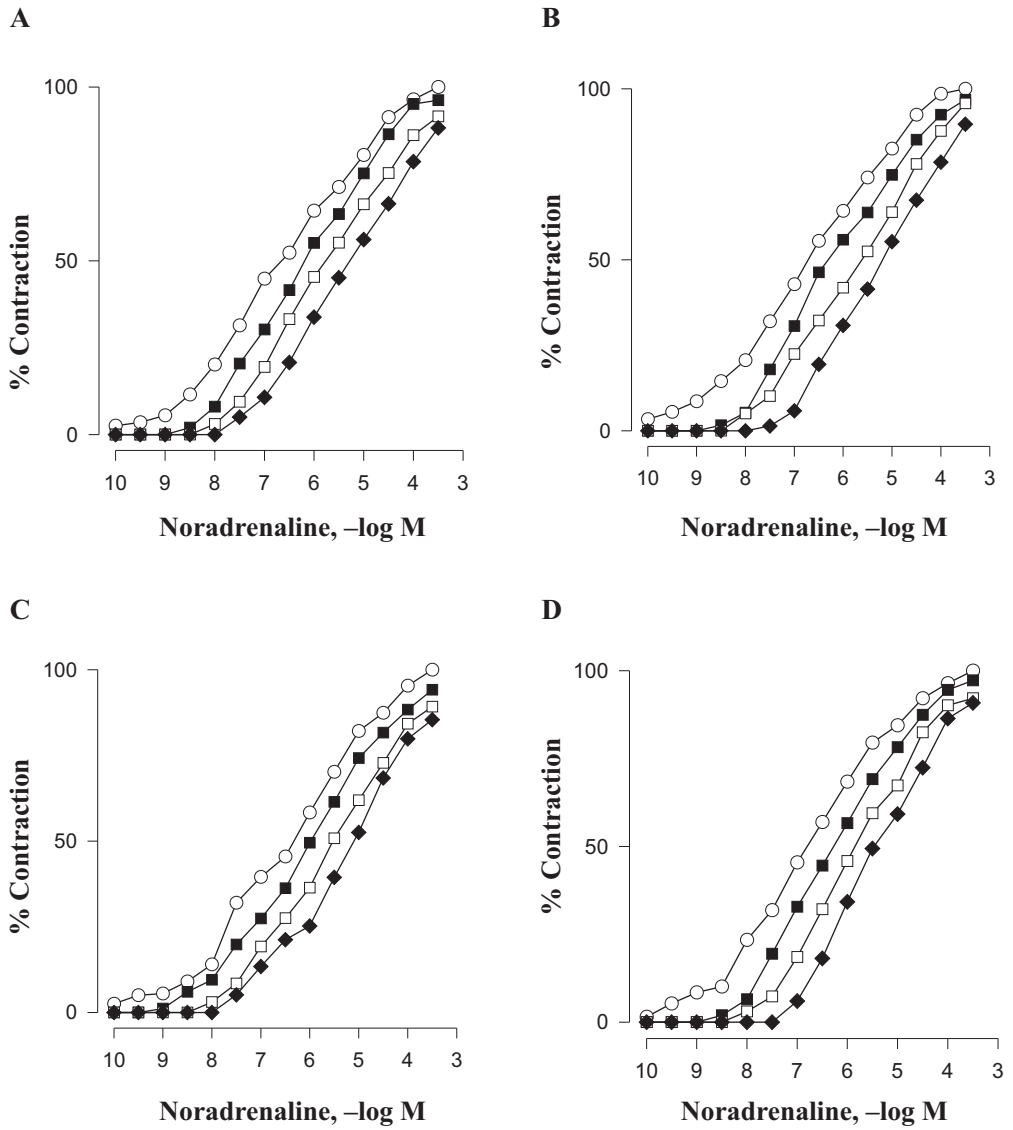

Fig. 4. Antagonism of the contractile effect of noradrenaline by antagonists of adrenergic receptors. Noradrenaline concentration-response curves in perforating branch of HIMA with intact (A) and denuded (B) endothelium in the absence (○) and presence of 4×10^{-10} (■), 1.3×10^{-9} (□) and 4×10^{-9} mol/l (◆) prazosin and 10^{-6} (■), 3×10^{-6} (□) and 10^{-5} mol/l (◆) rauwolscine (intact (C) and denuded (D) endothelium). Each point represents the mean of 4–12 experiments. SEM are excluded for clarity and do not exceed 15% of the mean value for each point. Responses are expressed as a percentage of the maximum contraction evoked by noradrenaline itself (3×10^{-4} mol/l)

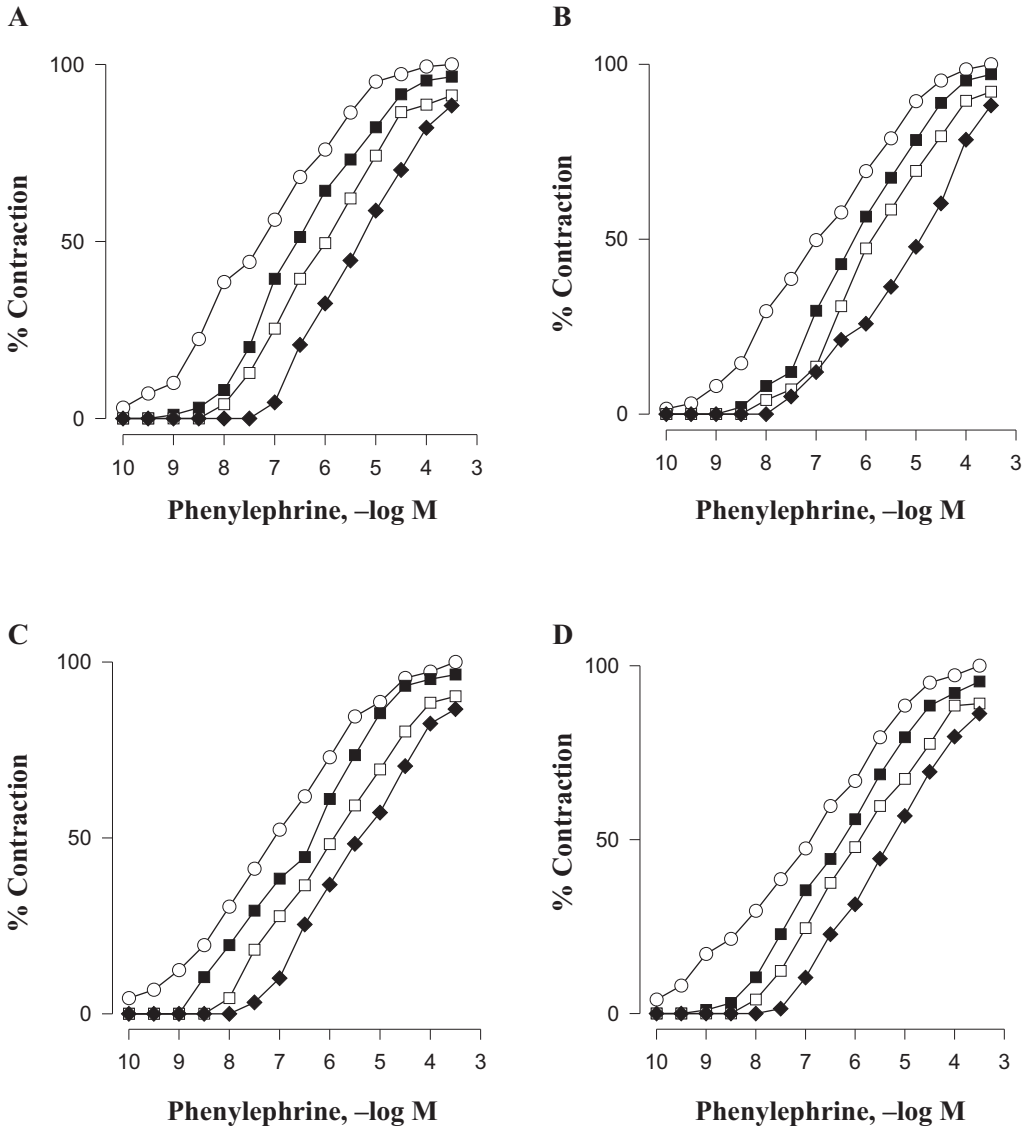

Fig. 5. Antagonism of the contractile effect of phenylephrine by antagonists of adrenergic receptors. Phenylephrine concentration-response curves in perforating branch of HIMA with intact (A) and denuded (B) endothelium in the absence (○) and presence of 4×10^{-10} (■), 1.3×10^{-9} (□) and 4×10^{-9} mol/l (◆) prazosin and 10^{-6} (■), 3×10^{-6} (□) and 10^{-5} mol/l (◆) rauwolscine (intact (C) and denuded (D) endothelium). Each point represents the mean of 4–12 experiments. SEM are excluded for clarity and do not exceed 15% of the mean value for each point. Responses are expressed as a percentage of the maximum contraction evoked by phenylephrine itself (3×10^{-4} mol/l)

intercept of the line with the abscissa was 9.96 ± 0.16 and 9.89 ± 0.25 for prazosin and 6.33 ± 0.04 and 6.37 ± 0.13 for rauwolscine effect on arterial rings with intact endothelium and denuded, respectively (Tab. 1, Fig. 6B).

DISCUSSION

In our previous studies, we demonstrated endothelium-dependent relaxation of the perforating branch of the HIMA, the role of NO and other endothelium derived factors [28] and defined the subtypes of muscarinic receptors on endothelium [27] and smooth muscle cells [26]. The results of our experiments showed that ACh provoked endothelium-dependent relaxation of the perforating branche of the HIMA, that was mediated, most likely, by NO synthesis and secretion from endothelial cells [28]. The aim of this study was the further explanation of adrenergic agonist action on the same artery.

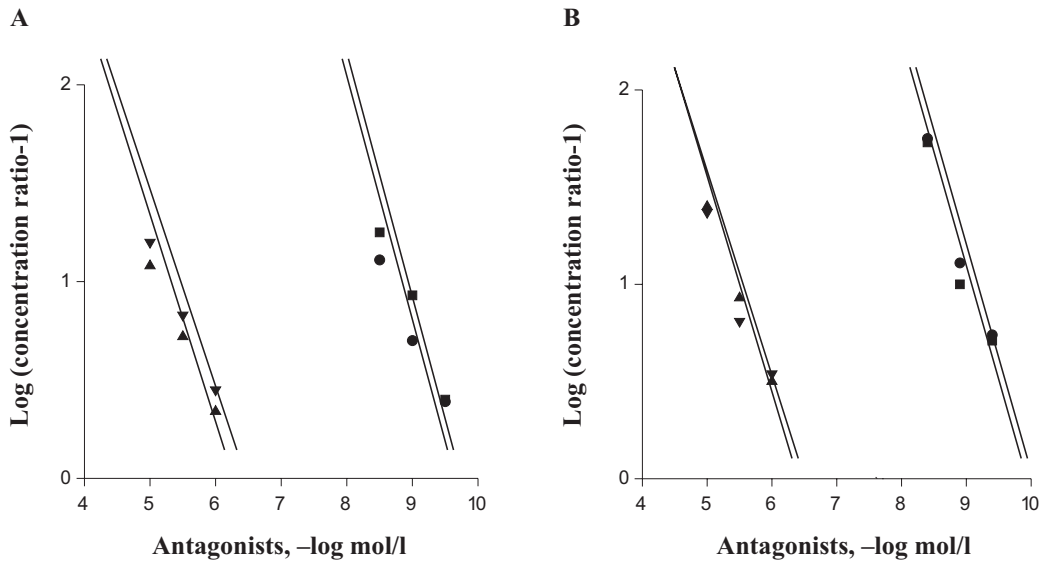

Fig. 6. Schild plot of log (concentration ratio-1) against –log [antagonist] for noradrenaline-prazosin (A) and phenylephrine-prazosin (B) antagonism on the isolated perforating branch of HIMA on endothelium intact (●) and denuded (■) preparations and noradrenaline-rauwolscine (A) and phenylephrine-rauwolscine antagonism (B) on endothelium intact (▲) and denuded (▼) preparations

Table 1. The pA_2 values, slopes of Schild plot and coefficients of correlation obtained for adrenoceptors antagonists in perforating branch of HIMA rings with intact and denuded endothelium

		pA_2	Slope	Correlation
Noradrenaline				
Endothelium intact				
	Prazosin	9.56 ± 0.03	0.913 ± 0.03	0.999
	Rauwolscine	6.11 ± 0.08	0.96 ± 0.1	0.994
Endothelium denuded				
	Prazosin	9.60 ± 0.15	1.065 ± 0.19	0.984
	Rauwolscine	6.31 ± 0.08	0.91 ± 0.09	0.996
Phenylephrine				
Endothelium intact				
	Prazosin	9.96 ± 0.16	1.09 ± 0.06	0.99
	Rauwolscine	6.33 ± 0.04	1.04 ± 0.04	0.999
Endothelium denuded				
	Prazosin	9.89 ± 0.25	1.10 ± 0.1	0.973
	Rauwolscine	6.37 ± 0.13	0.96 ± 0.13	0.991

In the present study we showed that noradrenaline (combined α and β stimulator) induced concentration-dependent contractions of perforating branch of the HIMA and that was consistant with the previous results obtained on the main arterial tree of HIMA [23, 31] and some other human arteries [15, 16]. The obtained pEC_{50} values were similar in preparations with and without endothelium and with those obtained in other blood vessels.

The presence of endothelium is known to modulate agonist-induced contractions of animal arteries. This has been particulary shown for noradre-

naline [8, 12, 22]. However, removal of the endothelium in our experiments did not affect responses of the perforating branch of the HIMA to noradrenaline. There was only a slight potentiation of contractions in denuded arterial segment, but pEC_{50} values were similar and without significant differences. This result was in contrast with previous findings obtained in resting HIMA that concentration-response curve of noradrenaline-induced contractions was significantly shifted to the left after endothelium rubbing, without changes in maximal responces. In unrubbed HIMA rings, the EC_{50} value of noradrenaline was about two-fold greater that in rubbed rings [23, 31]. Our results were similar to those obtained on human uterine, radial and renal artery [15, 16, 33] and was not consistent with the concept that the modulation of noradrenaline-induced contractile response by endothelium exists in all types of blood vessels, which is related to endothelium-derived relaxing factors release [2].

The noradrenaline-induced contraction in our experiments was not affected by hydrocortisone and desipramine, suggesting that only little neuronal and extraneuronal uptake occurs in the perforating branch of the HIMA.

Until now, there were only a few reports regarding the influence of endothelium on phenylephrine (specific α_1 stimulator)-induced contractions of human arteries. Some recent reports confirmed concentration-dependent contractions induced by phenylephrine, especially in microcirculation [40]. Our results showed that phenylephrine induced concentration-dependent contractions of perforating branch of the HIMA. Removal of endothelium did not change this action. The pEC_{50} value obtained for intact and endothelium-denuded arterial segments were similar. The sensitivity of perforating branch of HIMA to phenylephrine (based on the pEC_{50} values) was only slightly higher than to noradrenaline.

The pretreatment of both intact and denuded of endothelium arterial segments with indomethacin did not modify their sensitivity to phenylephrine or to noradrenaline indicating that products of cyclooxygenase patway have no effect on noradrenaline- and phenylephrine-induced contractions.

Contractions of the perforating branch of the HIMA with intact endothelium induced by noradrenaline were uneffected also by pretreatment with inhibitor of NO synthase, L-NMMA, suggesting that there is no basal NO secretion from endothelium modulating this effect. Similar results were obtained in arterial rings denuded of endothelium. Our results were consistent with those obtained on the human renal arteries [33] or porcine pulmonary artery [4].

Contrary, contractions of the perforating branch of the HIMA both intact and denuded of endothelium, induced by phenylephrine were modified by L-NMMA pre-aplication. L-NMMA provoked strong potentiation of phenylephrine-induced contraction of preparation with intact endothelium followed by increased maximal effect. The potentiation of contractions of arterial segments without endothelium was expressed only after high phenylephrine concentration (higher than 3×10^{-6} mol/l). The results obtained on arteries with intact endothelium could lead to the conclusion that the inhibition of basal NO secretion from endothelium by L-NMMA may be the reason of this potentiation. The results obtained on arterial segments denuded of endothelium and similar concentration-responses curves for phenylephrine before and after removal of endothelium exclude this possibility. The reason of potentiation of phenylephrine-induced contraction after L-NMMA application still remains unclear. We supposed that phenylephrine itself probably stimulated NO release from endothelium. We accepted as the criteria for endothelium denudation if relaxation to ACh was less than 50%. That means that we had no complete removal of endothelium in some preparations. It is possible that phenylephrine applicated at higher concentration stimulated also secretion of NO from this small part of intact endothelium even in preparations that we consider as denuded of endothelium. This hypothesis unfortunately remains for now unproveable. On the other hand, the effect of L-NMMA on NO-synthase in the blood vessels adventitia cannot also be excluded.

The possible influence of the ageing on phenylephrine-induced contractions was also reported. Therefore, the application of NO sythase inhibitor, L-NAME, shifted to the left the concentration-response curve for phenylephrine only in small mesenteric arteries from old but not from young rats [6].

It is established that noradrenaline induces contractions of isolated blood vessels through activation of both α_1- and α_2-adrenoceptors [36]. The treatment with propranolol did not affect the concentration-response curves for noradrenaline in the studied preparations. It appears that there is probably not a significant population of β-adrenoceptors in the perforating branch of the HIMA.

The pA_2 value for an antagonist in blocking the response to an agonist should be an accurate indication of its affinity for the receptor site if certain criteria were fulfilled [13, 21, 29]. In order to compare the contribution of different α-adrenoceptor subtypes to the noradrenaline- and phenylephrine-induced contraction, we used prazosin, a predominantly α_1-adrenoceptor antagonist [7, 34, 35] and rauwolscine, a predominantly α_2-adrenoceptor antagonist [10, 32, 38].

Although both antagonists are considered as competitive, Alosachie and Godfraind [1, 2] reported in the rat aorta non-competitive antagonism by prazosin of the noradrenaline concentration-response curve in the presence of endothelium and competitive antagonism in the absence of it. We did not observe this deviation of antagonism by prazosin since the slopes of Schild plot for both prazosin and rauwolscine were not significantly different from unity, indicating that the antagonism is competitive. In the perforating branch of the HIMA with intact endothelium affinity estimates for prazosin and rauwolscine were not different from those obtained in preparations denuded of endothelium. Therefore, the possibility that different α-adrenergic receptor subtypes are involved in noradrenaline-induced contraction of perforating branch of the HIMA with both intact endothelium and denuded of it was eliminated.

The affinities of prazosin for antagonizing the contractile action of noradrenaline was within the range reported for α_1-adrenoceptor blocade [34] which suggests the presence of contraction-mediating α_1-adrenoceptors in the studied preparations. In addition, pA_2 values for prazosin obtained in our study were similar to those reported previously for human saphenous vein [14] and different animals arteries [9, 17, 18] and higher than those obtained for human uterine artery [22] or main tree of HIMA [14].

In contrast, the pA_2 values for rauwolscine binding to receptors mediating contraction of perforating branch of the HIMA were not analogous to pA_2 values for α_2-adrenoceptors obtained in rabbit ear and saphenous vein [10, 32]. The obtained pA_2 values are similar to the affinity of postsynaptic α_1-adrenoceptor for rauwolscine in rabbit ear artery and aorta [9, 38]. These findings further proved the concept that there is only α_1-adrenoceptor in the perforating branch of the HIMA.

In some recent reports, contribution of α_1-adrenoceptor to noradrenaline-induced contractions of various blood vessels was also shown [12, 24, 39].

Similarly to noradrenaline, phenylephrine provoked concentration-dependent contractions of the perforating branch of the HIMA, stimulating only α_1-adrenoceptor. Antagonism was competitive (based on the slope of the Schild plot) in both preparations with intact and denuded of endothelium. The obtained pA_2 values for prazosin and rauwolscine in both preparations with and without endothelium were consistent with the affinity of α_1-adrenergic receptors for the used antagonists and with previously reported results obtained in different blood vessels [5, 24].

On the basis of these results, it seems reasonable to suggest that in perforating branch of the HIMA noradrenaline and phenylephrine induce contractions predominantly by activation of α_1-adrenoceptor, regardless of endothelial condition.

In conclusion, this study has shown that noradrenaline and phenylephrine induce concentration-dependent contractions of the perforating branch of the HIMA. Removal of the endothelium did not affect noradrenaline- and phenylephrine-induced contractions, suggesting a lack of endothelium-dependent modulation of their effects in the studied preparations. Products of cyclooxygenase pathway had no influence on noradrenaline and phenylephrine action. Endothelium-derived NO had no modulatory effect of noradrenaline-induced contractions, but inhibition of NO synthesis provoked potentiation of phenylephrine-induced contractions either in preparations with intact endothelium or denuded of it. The mechanism of this effect still remains unclear, but phenylephrine-induced NO secretion may be taken into consideration. On the basis of differential affinity for antagonists and for noradrenaline and phenylephrine themselves, we suggest that the identical subtype of α-adrenoceptors, probably α_1 subtype, is involved in the noradrenaline- and phenylephrine-induced contraction of the intact and endothelium-denuded perforating branch of the HIMA.

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