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# PHYSIOLOGICAL ANTAGONISM OF ANGIOTENSIN II AND LIPOPOLYSACCHARIDES IN EARLY ENDOTOXEMIA: PHARMACOMETRIC ANALYSIS

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The inhibitory effect of lipopolysaccharides (LPS) on α-adrenergic contraction is quite well known, but molecular mechanism of this inhibition is unclear. In the present study, the interaction between α-adrenoceptor and vasopressin receptor response, and LPS in rat tail artery was investigated using chemical stimulation. In the presence of LPS, noradrenaline, phenylephrine and arginine-vasopressin, concentration-response curves (CRCs) were shifted to the right with the change in maximal responses. The $K_A$ and $K_B$ values calculated in the presence and absence of LPS did not differ significantly. The results strongly suggest that LPS did not change the receptors affinity. The changes in the relationship between receptor occupancy and response to an agonist in the presence of LPS and reduction of $K_A/ED_{50}$ value suggest reduction of receptor reserve. In the presence of angiotensin II (Ang II), CRCs were shifted to the right with significant increase in receptor reserve. Moreover, this effect was still present in LPS-pretreated arteries. The receptor reserve reduced by LPS significantly increased in the presence of Ang II.

It suggests that inhibitory effect of LPS is partially reversible. The results strongly suggest that in early endotoxemia, inhibitory effect of LPS may by partially reverted by an increase in activity of renin-angiotensin-aldosterone system.

***Key words:*** *receptor reserve, septicemia, lipopolysaccharides, angiotensin II, nitric oxide, rat tail artery*

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*Abbreviations:* Ang II – *angiotensin II,* AVP – *arginine-vasopressin,* LPS – *lipopolysaccharide,* NA – *noradrenaline,* NO – *nitric oxide,* NOS – *nitric oxide synthase,* PHE – *phenylephrine*

## INTRODUCTION

The inhibitory effect of lipopolysaccharides (LPS) on α-adrenergic contraction in early endotoxemia is quite well documented, but molecular mechanism of this inhibition is still unclear. The function of adrenergic system in endotoxemia and septic shock was investigated in many experiments. The ability of the adrenergic system to elicit response in the arteries is one of the mechanisms of decreased vascular response to agonists of α-adrenergic and vasopressin receptors in septic shock. The attenuated activity of the adrenergic system, observed in endotoxemia, may be a result of central blood flow regulation [17] or peripheral regulation by local vasodilators [12].

Endotoxins and mediators released by endotoxins stimulate production of nitric oxide (NO) [3, 11, 16]. NO is a cell signaling molecule that has been demonstrated to be involved in a variety of physiological and pathophysiological processes. For example, NO is an important messenger involved in the regulation of the vascular smooth muscle tone, neurotransmission, inflammatory responses and host defense. NO is produced by at least two different types of enzymes, responsible for the formation of NO *in vivo*: inducible and constitutive nitric oxide synthases (iNOS and cNOS). iNOS was found in many cell types, such as macrophages and smooth muscle cells and it was shown to be induced by LPS and cytokines. iNOS requires β-nicotinamide adenine dinucleotide phosphate (β-NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide and tetrahydrobiopterin but not calcium and calmodulin as a cofactor for activation. It is believed that iNOS has a significant role in immune responses, as it has been implicated in killing tumor cells and microbes, development of inflammatory responses and reactions to septic shock. iNOS will produce NO continuously once it is induced. Two cNOS isoforms are known, one of which was found in endothelial cells (eNOS) and the other was found in the neurons (nNOS). Both isoforms require calcium, calmodulin and also β-NADPH, FAD, flavin mononucleotide and tetrahydrobiopterin as cofactors for activation. eNOS is primary involved in the regulation of blood vessel tone. NOS down- and up-regulation could have many significant effects in the mechanisms of a wide range of cardiovascular diseases, diabetes mellitus, renal function and reperfusion following vascular clamp release, and organ transplantation. Thus, vascular endothelium plays an important role as a metabolic and endocrine organ [30].

The activity of $\alpha_1$- and $\alpha_2$-adrenergic receptors depends on the distribution of these receptors in the vascular system [6, 23]. The larger arterioles, such as rat tail artery, have predominantly $\alpha_1$-adrenoceptors, whereas vessels of microcirculation express predominantly $\alpha_2$-adrenoceptors [8]. Mechanism of attenuation of α-adrenergic receptor function by changing the levels of G protein subunits is quite well known [27]. Some authors have shown altered G protein levels upon prolonged stimulation. Mechanism of short-term attenuation is still unclear.

NO is one of mediators of sepsis. Some results suggested that NO plays a significant role only in early sepsis. Attenuation of activity of α-adrenoceptors in the presence of LPS is concentration-dependent. Inhibition of adrenergic response is partially reversible in the presence of $N^{\omega}$-nitro-L-arginine methyl ester, an iNOS inhibitor [3, 11].

Angiotensin II (Ang II) is well known to enhance vasoconstrictor response to noradrenaline (NA) and phenylephrine (PHE). Ang II enhances the response of the isolated blood vessels to sympathetic nerves [26, 31]. It elevates the response of blood vessels and other organs to electrical stimulation of sympathetic nerves resulting in an increased release of adrenergic neurotransmitters [2, 7]. Ang II increases the release of NA from adrenal glands [18]. At subtherapeutic doses, Ang II also enhances response to the application of endogenous and exogenous catecholamines [32, 34].

Results of early experiments performed in our laboratory suggest that the loss of vascular tone may be a direct result of reduction of α-adrenergic receptor reserve. We have also reported that down-regulation of α-adrenergic receptor reserve is observed in the presence of 8Br-cGMP while down-regulation of angiotensin type 1 receptors may occur in the presence of NO [4]. Another phenomenon, namely up-regulatory effects were noted for example in the presence of Ang II. The main aim of this study was to determine the role of Ang II in the mechanisms of regulation of vascular tone during

early endotoxemia, i.e. within the first minutes and hours.

## MATERIALS and METHODS

The experiments were performed on male Wistar rats, weighing 220–270 g. Animals were anesthetized with urethane (120 mg/kg, *ip*). Tail artery was excised and gently cleaned off adherent tissue. The proximal segment (2–3 cm) of the rat tail artery was cannulated and mounted under 0.5 g tension in an 20 ml organ bath [20, 22]. During the first stage of the experiments, all arteries were stabilized in Krebs solution (pH 7.4, 37°C) by gradually increasing perfusion rate from 0.25 to 1.0 ml/min until perfusion pressure between 2–4 kPa was reached (about 2 h). The constriction of the tail artery in response to NA, PHE and arginine-vasopressin (AVP) was measured as an increase in perfusion pressure at a constant flow of the perfusion fluid (about 1 ml/min). Vasoconstrictor drugs as well as LPS, Ang II were applied into the extraluminal Krebs solution. Cumulative response curves ($CRC_S$) to agonists were obtained using Van Rossum method [29]. Registration system was composed of physiological pressure transducer (Statham-Gould type P23ID), perfusion pump (ZALIMP, type 315) and polyphysiograph (Narco Bio Systems Inc. – Narcotrace 40).

### Analysis of results

The NA, PHE and AVP concentration necessary to achieve half-maximal contraction ($EC_{50}$) was calculated by linear regression of the 20–80% region of each concentration-response curve. $pD_2$ values were calculated as the negative logarithm of the $EC_{50}$ value. Results were related to the values obtained in control arteries. $pK_B$ values for antagonists were calculated using classical Arunlakshana and Schild method. Schild plots, drawn by linear regression, were constructed as log (dose ratio – 1) plotted against logarithm of antagonist concentration. Dose ratios (dr) values were calculated at the $EC_{50}$ levels. Antagonism was considered to be competitive when slope of the Schild plot did not differ from unity. The affinity of agonists ($K_A$ values) was calculated using the method of Furchgott and Bursztyn. $K_A$ values were used to calculate the fraction of receptors occupied ($[RA]/[R_T]$). For more details see Kenakin [15].

### Drugs and solutions

Drugs used in the experiments were purchased from Sigma Chemical Company. Krebs solution was composed of KCl (4.7 mM), $MgSO_4$ (1.2 mM), $KH_2PO_4$ (1.2 mM), NaCl (117.8 mM), $NaHCO_3$ (26.4 mM), glucose (11.1 mM) and $CaCl_2$ (2.3 mM) at pH 7.4. To eliminate effects mediated by $\alpha_2$-adrenoceptors, yohimbin ($3 \times 10^{-9}$ M) was added.

### Data analysis

The results are means ± SEM. Statistical analysis was performed using the Newman-Keuls test for multiple comparison of means; $p < 0.05$ was considered as statistically significant.

## RESULTS

$CRC_S$ for NA ($10^{-10} – 3 \times 10^{-4}$ M), a non-selective α-adrenoceptor agonist, PHE ($10^{-9} – 10^{-3}$ M), a preferential $\alpha_1$-adrenoceptor agonist and AVP ($10^{-10} – 10^{-4}$ M), a non-selective vasopressin receptor agonist, were compared in the absence and in presence of LPS (2.5 μg/cm$^3$, 0.25 μg/cm$^3$, 0.025 μg/cm$^3$, incubation for 120 min). In the presence of LPS, $CRC_S$ were shifted to the right with the change in maximal responses. The $CRC_S$ obtained for NA, PHE and AVP in the presence of Ang II were shifted to the left without any change in maximal responses (Fig. 1A, B, C). Comparison of $EC_{50}$ values indicated that treatment of the segments with LPS significantly reduced the affinity of NA and PHE for α-adrenoceptors and AVP for vasopressin receptors, whereas in the presence of Ang II, the affinity of NA, PHE and AVP for its receptors significantly increased (Tab. 1, 3 and 5).

Prazosin at concentrations of $10^{-8}$ M, $3 \times 10^{-8}$ M and $10^{-7}$ M produced a concentration-dependent, parallel shift of the $CRC_S$ for NA without any change in maximal responses in absence and in presence of LPS (0.25 μg/cm$^3$, incubation for 90 min). The slope of the Schild plot did not differ significantly from unity indicating competitive antagonism. Calculated $pK_B$ values were 9.70 (± 0.10) and 9.72 (± 0.09) in the absence and in the presence of LPS, respectively. Values did not differ significantly.

The control $K_A$ values obtained for NA, PHE and AVP in the absence of LPS were 1.50 (± 0.76) $\times 10^{-7}$, 3.38 (± 0.54) $\times 10^{-7}$ and 1.21 (± 0.44) $\times 10^{-7}$, respectively. The $K_A$ values for NA, calculated

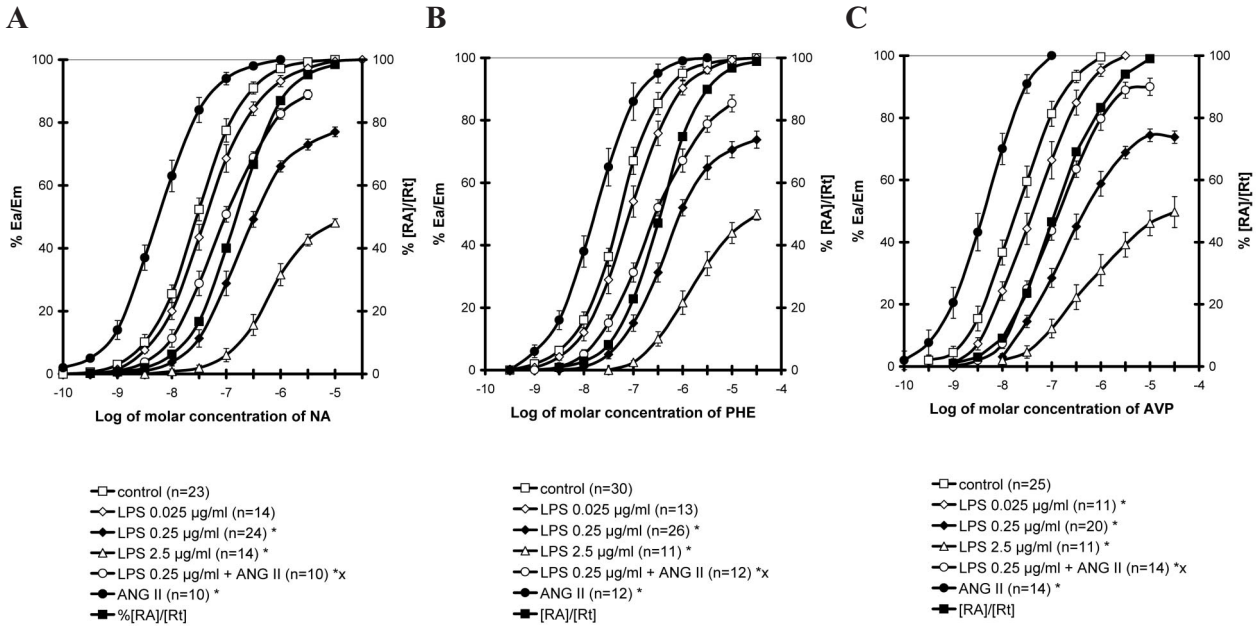


*Fig. 1.* Effects of Ang II on concentration-response curve for (**A**) noradrenaline (NA), (**B**) phenylephrine (PHE) and (**C**) arginine-vasopressin (AVP) in LPS-pretreated arteries. Each point represents the mean and vertical lines show SEM. * $p < 0.05$ vs. control, $^{\times}$ $p < 0.05$ vs. LPS (0.25 µg/ml)-pretreated artery

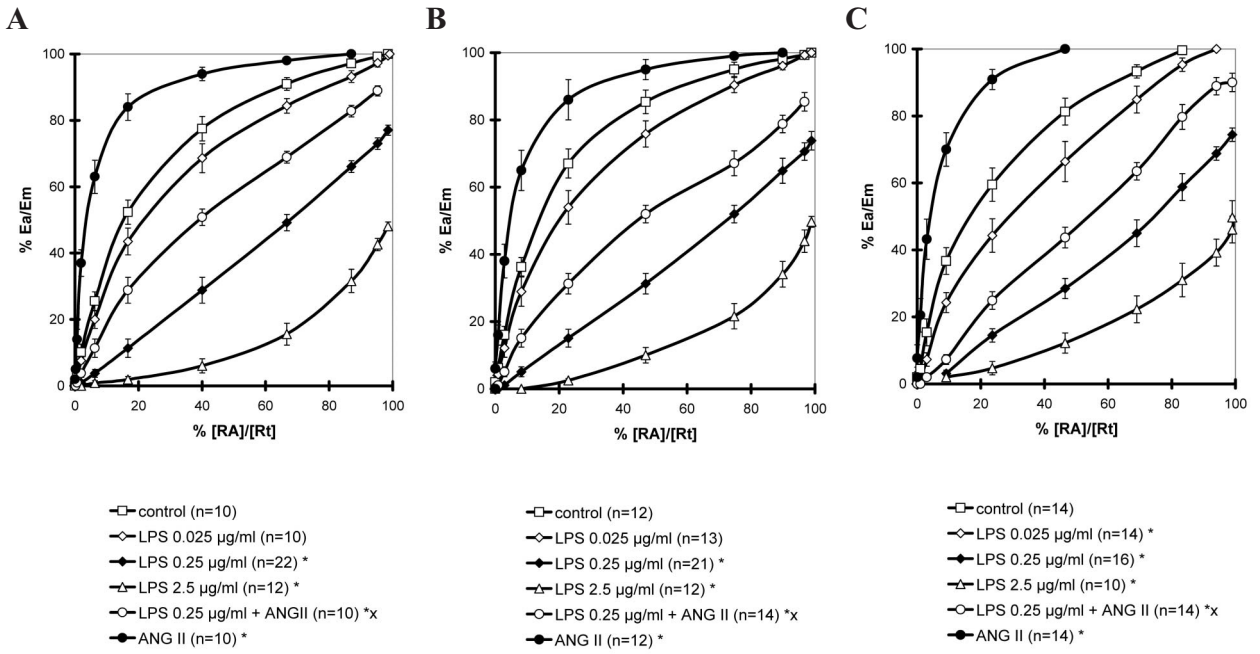


*Fig. 2.* Effects of Ang II on agonist responses vs. receptor occupancy plots for (**A**) noradrenaline (NA), (**B**) phenylephrine (PHE) and (**C**) arginine-vasopressin (AVP) in LPS-pretreated arteries. Each point represents the mean and vertical lines show SEM. * $p < 0.05$ vs. control, $^{\times}$ $p < 0.05$ vs. LPS (0.25 µg/ml)-pretreated artery

in the presence of LPS (2.5 μg/cm$^3$, 0.25 μg/cm$^3$, 0.025 μg/cm$^3$) were 1.60 (± 0.78) × $10^{-7}$, 1.63 (± 0.48) × $10^{-7}$, 1.41 (± 0.55) × $10^{-7}$, respectively. The $K_A$ values obtained for PHE in the presence of LPS were 3.77 (± 0.88) × $10^{-7}$, 3.60 (± 0.63) × $10^{-7}$, 3.31 (± 0.68) × $10^{-7}$, respectively. In the presence of LPS, the $K_A$ values for AVP were 1.52 (± 0.96) × $10^{-7}$, 1.20 (± 0.75) × $10^{-7}$ and 1.11 (± 0.98) × $10^{-7}$, respectively. The $K_A$ values for NA, PHE and AVP determined in the presence of various concentrations of LPS did not differ significantly. Relative potency and relative efficacy decreased in the presence of LPS but relative affinity did not change in the presence of LPS. Relative potency, relative affinity and relative efficacy calculated for NA, PHE and AVP also did not significantly change during incubation (30 min) in the presence of Ang II ($10^{-9}$ M) with LPS (Tab. 1–6). In the presence of losartan (Ang II type 1 receptor antagonist), the intensification effect of Ang II was inhibited (Tab. 1–6).

Agonist responses vs. receptor occupancy curves for NA, PHE and AVP were compared in the ab-

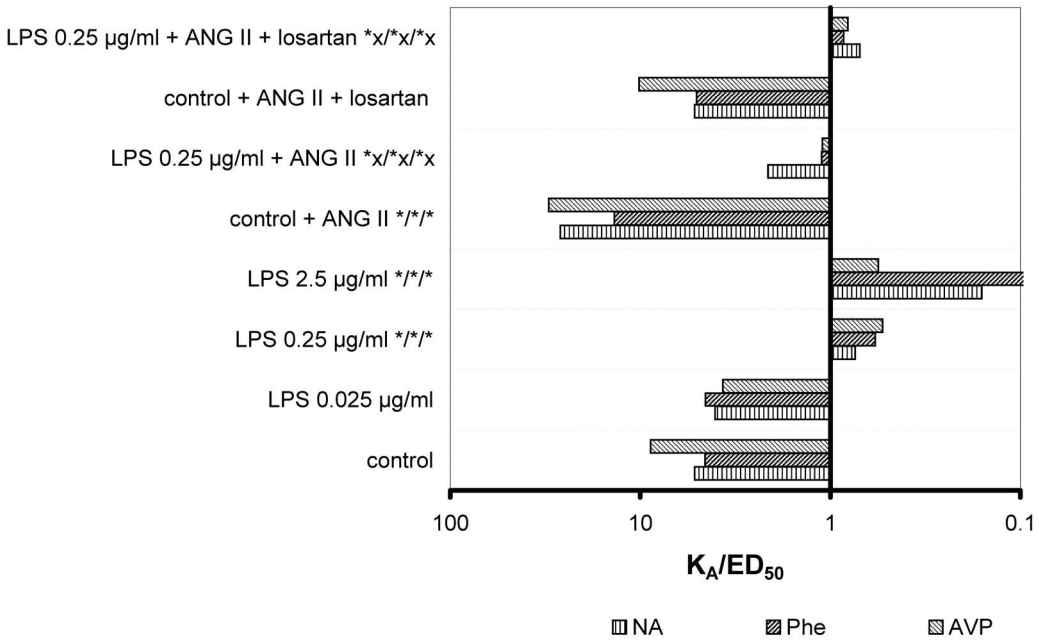


*Fig. 3.* Effect of Ang II on $K_A/ED_{50}$ for noradrenaline (NA), phenylephrine (PHE) and arginine-vasopressin (AVP) (p-values for NA/Phe/AVP), * $p < 0.05$ vs. control (NA, PHE or AVP), $^{\times}$ $p < 0.05$ vs. LPS (0.25 μg/ml)-pretreated artery

*Table 1.* $EC_{50}$ and relative potency for noradrenaline (NA) in the absence and presence of LPS and in the absence and presence of Ang II

| | n[a] | %$E_{max}$ [b] | $EC_{50}$ [M] | $pD_2$ | RP[c] |
|---|---|---|---|---|---|
| In absence of LPS | 23 | 100 | 2.90 (± 0.70) × $10^{-8}$ | 7.54 | 100% |
| LPS 0.025 μg/cm$^3$ | 14 | 100 | 3.96 (± 0.95) × $10^{-8}$ | 7.40 | 73% |
| LPS 0.25 μg/cm$^3$ | 24 | 77 | 2.20 (± 1.10) × $10^{-7}$* | 6.66 | 13% |
| LPS 2.5 μg/cm$^3$ | 14 | 48 | 8.78 (± 1.68) × $10^{-7}$* | 6.06 | 3% |
| Control + Ang II | 10 | 100 | 5.42 (± 1.32) × $10^{-9}$* | 8.27 | 535% |
| LPS 0.25 μg/cm$^3$ + Ang II | 10 | 90 | 8.25 (± 1.27) × $10^{-8}$*$^{\times}$ | 7.08 | 35% |
| Control + Ang II + losartan | 8 | 100 | 2.78 (± 0.92) × $10^{-8}$ | 7.55 | 104% |
| LPS 0.25 μg/cm$^3$ + Ang II + losartan | 8 | 80 | 2.10 (± 1.37) × $10^{-7}$* | 6.78 | 14% |

[a] n – number of $CRC_S$ used in calculation, [b] %$E_{max}$ – % of maximal perfusion pressure, [c] RP – relative potency – calculated in reference to the $EC_{50}$ of NA; * $p < 0.05$ vs. control (NA), $^{\times}$ $p < 0.05$ vs. LPS (0.25 μg/ml)-pretreated artery

*Table 2.* Comparison of $K_A$ values, relative affinities, relative efficacies for noradrenaline (NA) in the absence and presence of LPS and in the absence and presence of Ang II

| | n[a] | $K_A$ [M] | $pK_A$ | $K_A/ED_{50}$ | RA[b] | RE[c] |
|---|---|---|---|---|---|---|
| In absence of LPS | 10 | $1.50 (\pm 0.76) \times 10^{-7}$ | 6.82 | 5.17 | 100% | 100% |
| LPS 0.025 μg/cm$^3$ | 10 | $1.60 (\pm 0.78) \times 10^{-7}$ | 6.80 | 4.04 | 94% | 72% |
| LPS 0.25 μg/cm$^3$ | 22 | $1.63 (\pm 0.48) \times 10^{-7}$ | 6.79 | 0.74* | 92% | 22% |
| LPS 2.5 μg/cm$^3$ | 12 | $1.41 (\pm 0.55) \times 10^{-7}$ | 6.85 | 0.16* | 106% | 7%❄ |
| Control + Ang II | 10 | $1.43 (\pm 0.89) \times 10^{-7}$ | 6.84 | 26.38* | 104% | 520% |
| LPS 0.25 μg/cm$^3$ + Ang II | 10 | $1.76 (\pm 0.80) \times 10^{-7}$ | 6.75 | 2.13*× | 85% | 64% |
| Control + Ang II + losartan | 8 | $1.52 (\pm 0.96) \times 10^{-7}$ | 6.82 | 5.17 | 100% | 100% |
| LPS 0.25 μg/cm$^3$ + Ang II + losartan | 8 | $1.48 (\pm 1.16) \times 10^{-7}$ | 6.83 | 0.70* | 101% | 23% |

[a] n – number of experiments, [b] RA – relative affinity – calculated in reference to $K_A$ in the absence of LPS, [c] RE – relative efficiency – with respect to NA at 50% of the maximal response. ❄ – Because of relative response < 50%, RE was calculated at a relative response of 30%; * $p < 0.05$ vs. control (NA), × $p < 0.05$ vs. LPS (0.25 μg/ml)-pretreated artery

*Table 3.* $EC_{50}$ and relative potency for phenylephrine (PHE) in the absence and presence of LPS and in the absence and presence of Ang II

| | n[a] | $\%E_{max}$ [b] | $EC_{50}$ [M] | $pD_2$ | RP[c] |
|---|---|---|---|---|---|
| In absence of LPS | 30 | 100 | $7.42 (\pm 0.76) \times 10^{-8}$ | 7.13 | 100% |
| LPS 0.025 μg/cm$^3$ | 13 | 100 | $8.30 (\pm 0.90) \times 10^{-8}$ | 7.08 | 89% |
| LPS 0.25 μg/cm$^3$ | 26 | 75 | $6.20 (\pm 1.12) \times 10^{-7}$* | 6.21 | 12% |
| LPS 2.5 μg/cm$^3$ | 11 | 50 | $1.53 (\pm 0.94) \times 10^{-6}$* | 5.82 | 5% |
| Control + Ang II | 12 | 100 | $3.24 (\pm 0.64) \times 10^{-8}$* | 7.49 | 229% |
| LPS 0.25 μg/cm$^3$ + Ang II | 12 | 86 | $3.04 (\pm 0.52) \times 10^{-7}$*× | 6.37 | 18% |
| Control + Ang II + losartan | 8 | 100 | $6.50 (\pm 1.10) \times 10^{-8}$ | 7.19 | 114% |
| LPS 0.25 μg/cm$^3$ + Ang II + losartan | 8 | 77 | $4.85 (\pm 0.86) \times 10^{-7}$* | 6.31 | 15% |

[a] n – number of $CRC_S$ used in calculation, [b] $\%E_{max}$ – % of maximal perfusion pressure, [c] RP – relative potency – calculated in reference to the $EC_{50}$ of PHE; * $p < 0.05$ vs. control (PHE), × $p < 0.05$ vs. LPS (0.25 μg/ml)-pretreated artery

sence and presence of LPS. In the absence of LPS the plots were hyperbolic. After incubation in the presence of LPS they became linear (Fig. 2A, B, C). Reductions of $K_A/ED_{50}$ values in the presence of LPS were observed too. Moreover, in LPS-pretreated arteries, in the presence of Ang II, the plots for NA, PHE and AVP were reversed from linear to hyperbolic indicating an increase in the receptor reserve. (Tab. 2, 4, 6 and Fig. 3).

## DISCUSSION

During the septic response, exposure to LPS activates a series of biochemical responses. In arteries of the isolated blood-perfused rat lungs, the biphasic reaction of arteries was observed. Biphasic reaction was not seen in the lungs perfused with plasma or Krebs buffer, suggesting important, protective role of morphotic elements of blood in production of mediators of sepsis, like NO [5, 9]. To eliminate donors of sepsis mediators other than vascular endothelium and smooth muscle cells, we perfused artery with Krebs buffer. Cellular response to LPS include the release of endogenous substances, such as cytokines, metabolites of arachidonic acid, coagulation factors, NO and others from endothelial cells, smooth muscle and immune defense system cells [9, 10, 33]. Some of these substances may be responsible for loss of vascular tone. In experimental sepsis *in vivo*, induced by

*Table 4.* Comparison of $K_A$ values, relative affinities, relative efficacies for phenylephrine (PHE) in the absence and presence of LPS and in the absence and presence of Ang II

| | n[a] | $K_A$ [M] | $pK_A$ | $K_A/ED_{50}$ | RA[b] | RE[c] |
|---|---|---|---|---|---|---|
| In absence of LPS | 12 | $3.38 (\pm 0.54) \times 10^{-7}$ | 6.62 | 4.56 | 100% | 100% |
| LPS 0.025 μg/cm$^3$ | 13 | $3.77 (\pm 0.88) \times 10^{-7}$ | 6.42 | 4.54 | 90% | 68% |
| LPS 0.25 μg/cm$^3$ | 21 | $3.60 (\pm 0.63) \times 10^{-7}$ | 6.44 | 0.58* | 94% | 20% |
| LPS 2.5 μg/cm$^3$ | 12 | $3.31 (\pm 0.68) \times 10^{-7}$ | 6.48 | 0.02* | 102% | 10%❄ |
| Control + Ang II | 12 | $4.45 (\pm 0.90) \times 10^{-7}$ | 6.35 | 13.73* | 76% | 208% |
| LPS 0.25 μg/cm$^3$ + Ang II | 14 | $3.62 (\pm 0.62) \times 10^{-7}$ | 6.44 | 0.85*× | 93% | 47% |
| Control + Ang II + losartan | 8 | $3.28 (\pm 0.83) \times 10^{-7}$ | 6.48 | 5.05 | 103% | 100% |
| LPS 0.25 μg/cm$^3$ + Ang II + losartan | 8 | $4.11 (\pm 0.96) \times 10^{-7}$ | 6.39 | 0.85* | 82% | 21% |

[a] n – number of experiments, [b] RA – relative affinity – calculated in reference to $K_A$ in the absence of LPS, [c] RE – relative efficiency – with respect to PHE at 50% of the maximal response. ❄ – Because of relative response < 50%, RE was calculated at a relative response of 40%; * $p < 0.05$ vs. control (PHE), × $p < 0.05$ vs. LPS (0.25 μg/ml)-pretreated artery

*Table 5.* $EC_{50}$ and relative potency for arginine-vasopressin (AVP) in the absence and presence of LPS and in the absence and presence of Ang II

| | n[a] | $\%E_{max}$ [b] | $EC_{50}$ [M] | $pD_2$ | RP[c] |
|---|---|---|---|---|---|
| In absence of LPS | 25 | 100 | $1.37 (\pm 0.36) \times 10^{-8}$ | 7.86 | 100% |
| LPS 0.025 μg/cm$^3$ | 11 | 100 | $4.12 (\pm 1.25) \times 10^{-8}$* | 7.38 | 34% |
| LPS 0.25 μg/cm$^3$ | 20 | 78 | $1.89 (\pm 1.12) \times 10^{-7}$* | 6.72 | 7% |
| LPS 2.5 μg/cm$^3$ | 11 | 48 | $3.17 (\pm 0.85) \times 10^{-7}$* | 6.50 | 4% |
| Control + Ang II | 14 | 100 | $4.24 (\pm 0.63) \times 10^{-9}$* | 8.37 | 323% |
| LPS 0.25 μg/cm$^3$ + Ang II | 14 | 92 | $1.44 (\pm 0.66) \times 10^{-7}$*× | 6.84 | 10% |
| Control + Ang II + losartan | 8 | 100 | $1.18 (\pm 1.28) \times 10^{-8}$ | 7.93 | 116% |
| LPS 0.25 μg/cm$^3$ + Ang II + losartan | 8 | 79 | $1.56 (\pm 0.88) \times 10^{-7}$* | 6.81 | 9% |

[a] n – number of $CRC_S$ used in calculation, [b] $\%E_{max}$ – % of maximal perfusion pressure, [c] RP – relative potency – calculated in reference to the $EC_{50}$ of AVP; * $p < 0.05$ vs. control (AVP), × $p < 0.05$ vs. LPS (0.25 μg/ml)-pretreated artery

*Table 6.* Comparison of $K_A$ values, relative affinities, relative efficacies for arginine-vasopressin (AVP) in the absence and presence of LPS and in the absence and presence of Ang II

| | n[a] | $K_A$ [M] | $pK_A$ | $K_A/ED_{50}$ | RA[b] | RE[c] |
|---|---|---|---|---|---|---|
| In absence of LPS | 14 | $1.21 (\pm 0.44) \times 10^{-7}$ | 6.92 | 8.83 | 100% | 100% |
| LPS 0.025 μg/cm$^3$ | 14 | $1.52 (\pm 0.96) \times 10^{-7}$ | 6.82 | 3.69* | 79% | 33% |
| LPS 0.25 μg/cm$^3$ | 16 | $1.20 (\pm 0.75) \times 10^{-7}$ | 6.92 | 0.53* | 66% | 17% |
| LPS 2.5 μg/cm$^3$ | 10 | $1.11 (\pm 0.98) \times 10^{-7}$ | 6.95 | 0.56* | 69% | 9%❄ |
| Control + Ang II | 14 | $1.15 (\pm 0.51) \times 10^{-7}$ | 6.94 | 30.35* | 105% | 320% |
| LPS 0.25 μg/cm$^3$ + Ang II | 14 | $1.48 (\pm 0.73) \times 10^{-7}$ | 6.83 | 1.02*× | 82% | 25% |
| Control + Ang II + losartan | 8 | $1.20 (\pm 1.25) \times 10^{-7}$ | 6.92 | 10.17 | 101% | 100% |
| LPS 0.25 μg/cm$^3$ + Ang II + losartan | 8 | $1.26 (\pm 0.72) \times 10^{-7}$ | 6.90 | 0.81* | 96% | 17% |

[a] n – number of experiments, [b] RA – relative affinity – calculated in reference to $K_A$ in the absence of LPS, [c] RE – relative efficiency – with respect to AVP at 50% of the maximal response. ❄ – Because of relative response < 50%, RE was calculated at a relative response of 30%; * $p < 0.05$ vs. control (AVP), × $p < 0.05$ vs. LPS (0.25 μg/ml)-pretreated artery

prolonged exposure to LPS, mortality may be reduced by metabolic fragment of bradykinin, Arg-Pro-Pro-Gly-Phe. In experiments performed on aortic smooth muscle pretreated with that metabolic fragment of bradykinin, reactivity of the tissue was also increased [21].

To further validate the concept that $\alpha_1$-adrenoceptor activity was significantly attenuated by endotoxemia, the responses to PHE and NA were tested. We have demonstrated that α-adrenergic receptor reserve is decreased in the presence of LPS. The changes in agonist response vs. receptor occupancy curves and reduction of $K_A/ED_{50}$ values strongly suggest reduction of receptor reserve in the presence of LPS. It is known that the main defect existing in endotoxemia is the ability of the adrenergic system to respond [1]. Baker et al. [1] have suggested that the reduced adrenergic vasoconstrictor response of cremaster muscle arterioles during endotoxemia is a result of greatly attenuated activity of $\alpha_2$-adrenergic receptors. The arteries tested by Baker et al. belong to small resistance arteries. Thus, there are arteries with predominantion of $\alpha_2$-adrenoceptors, whereas the rat tail artery is one of quite big resistance arteries with predominanting $\alpha_1$-adrenergic receptors. After the administration of LPS, the $ED_{50}$ values for NA and PHE were significantly decreased, indicating that the sensitivity of the receptors had greatly decreased. The loss of adrenergic reactivity may be a result of the changes in the affinity of receptor and agonist, or the decreasing receptor reserve.

The overproduction of NO due to induction of iNOS may be responsible for cardiac and vascular dysfunction in endotoxemia [13]. Some substances, such as NO, may decrease receptor reserve. Results of experiments performed in the presence of L-NAME suggest that this effect may be partially reversible. Thus, treatment with L-NAME or other NOS inhibitor should increase receptor reserve and increase perfusion pressure. Significant induction of iNOS in the rat aorta has been reported at 60 min after LPS administration [28]. Olsson et al. [24] reported an increase in the production of NO and cGMP in the rat urinary bladder after intraperitoneal injection of *Escherichia coli* LPS. MacMicking et al. [19] reported the decrease in death rates in iNOS deficient mice. Our experiments performed on the isolated rat tail artery with and without endothelium suggest that endothelium is necessary for inhibitory effect of LPS to occur. In experiments performed on the rat tail arteries without vascular endothelium, inhibitory effect of LPS was not statistically significant. Moreover, in LPS-pretreated arteries, in the presence of L-NAME, NOS inhibitor, the plots showing dependence of response to NA and PHE on receptor occupancy were reversed from linear to hyperbolic relation indicating an increase in the receptor reserve. The changes in these curves and reduction of $K_A/ED_{50}$ values strongly suggest reduction of receptor reserve in the presence of LPS. It may suggest that, in early endotoxemia, inhibitory effect of LPS is partially reversible after administration of inhibitors of NOS. It has also been reported that NOS inhibitor augmented the α-adrenoceptor response. The effect of inhibition of NOS by aminoguanidine (NOS inhibitor) in endotoxemia was described by Hock et al. [13]. In addition Kazmierski et al. [14] reported that binding of NO by iron chelates might decrease mortality in experimental models of septic shock. It shows important role of NO in early sepsis. Seasholtz et al. [27] suggested that desensitization of $\alpha_1$-adrenergic receptors in the aortic smooth muscles after exposure to catecholamines was directly connected with the changes in the levels of G-protein units. This mechanism may mediate the loss of function of not only $\alpha_1$-adrenoceptors but probably also other G-protein-coupled receptors. This effect was observed only after prolonged incubation. Seasholtz et al. [27] incubated rat aortic rings for 22 h. The changes in G-protein levels were observed also by Patten et al. [25] during 24 and 72 h long experimental sepsis. No change in G-protein levels was noted upon shorter incubation. The experiments with short incubation in the presence of LPS showed that inhibitory effect of LPS in isolated artery in the first hours of sepsis was predominantly a result of release of NO from endothelial cells and decrease in signal transduction between α-adrenoceptor and calcium channel. Thus, the changes in activity of enzymes, such as phospholipase C or protein kinase C or alterations of G-protein levels may mediate the loss of activity of α-adrenoceptors in the presence of LPS during prolonged sepsis, but in early septic shock, uncoupling receptor from their G-protein or uncoupling between G-protein and enzymes seems to be more important [11]. The presence of Ang II may partially reverse this process by increasing receptor reserve, thus, the physiological antagonism between

Ang II and LPS can be observed during early sepsis in isolated artery.

In conclusion, our results strongly suggest that physiological antagonism between LPS and Ang II is predominantly a resultant of decreasing signal transduction by LPS-induced NO release from vascular endothelium and increasing signal transduction by Ang II-induced enhancement of the receptor reserve. Both, LPS and Ang II, act on the pathway between receptor and calcium channel. Thus, the changes in activity of enzymes such as phospholipase C or protein kinase C or alterations of G-protein levels may mediate the loss of function of receptors in the presence of LPS in prolonged sepsis, but in early septic shock, uncoupling of receptor from their G-protein or uncoupling between G-protein and enzymes seems to be more important. Moreover, this process seems to be partially reversible, thus, treatment with substances increasing the receptor reserve may be effective in early sepsis.

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