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ROLE OF NITRIC OXIDE IN RAT KIDNEY VASCULAR BED IN ENDOTOXEMIA*

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***** Bu makale bir uzmanlık tezinden türetilmiştir.

ENDOTOKSEMİDE SIÇAN BÖBREK VASKÜLER YATAĞINDA NİTRİK OKSİTİN ROLÜ

ÖZET

Sepsisin patofizyolojisi ve tedavi seçenekleri üzerinde yapılan çalışmalar sitokinler ve mediyatörler üzerinde yoğunlaşmıştır. İlk kez endotel kaynaklı gevşetici faktör olarak adlandırılan ve sonrasında nitrik oksit (NO) olduğu saptanan mediyatörün bu süreçte önemli olabileceği düşünülmüştür. Çalışmamızda endotoksemide böbrek vasküler yatağında meydana gelen değişiklikler ve L-arjinin:NO yolağının bu süreçteki etkisinin araştırılması hedeflenmiştir. Çalışma sıçanlardan hazırlanan izole perfüze böbrek preparatlarında gerçekleştirilmiştir. Kontrol grubuna 4 saat önceden intraperitoneal serum fizyolojik injeksiyonu yapılırken endotoksemi grubuna LPS (20mg/kg;ip) uygulanmıştır. Endotoksemi grubunda bazal perfüzyon basıncında ve fenilefrin (0.1-3 µM)'e verilen kasıcı cevaplarda anlamlı fark saptanmadı. Endotoksemi grubunda asetil kolin (0.1-1000 µg)'e verilen endotele bağlı gevşeme cevaplarının anlamlı olarak azaldığı gösterildi.

Aminoguanidin (20mg/kg; ip; 4 saat önce) uygulaması asetilkolin (0.1-1000 µg)'e verilen endotele bağlı gevşeme cevaplarını kontrol grubunda değiştirmezken endotoksemi grubunda azalmış endotele bağlı gevşeme cevaplarını tamamen düzeltmiş ve kontrol düzeylerine yükseltmiştir. LPS'in akut etkisini saptamayı hedefleyen bölümde ise perfüzyon solüsyonuna eklenen LPS bazal perfüzyon basıncını değiştirmedi. Fenilefrin ile perfüzyon basıncının arttırılmasından sonra LPS'in perfüzyon basıncında 3µg/ml ve 10µg/ml

konsantrasyonlarda, akut, konsantrasyona bağlı, L-NAME (30 μM) ile inhibe edilen ve tekrarlanmayan bir düşüş sağladığı gösterildi.

Endotoksemi grubunda bazal perfüzyon basıncında ve fenilefrine verilen kasıcı cevaplarda anlamlı fark saptanmaması endotoksemiye bağlı artmış nitrik oksit üretimine ikincil olarak böbrek mikrovasküler yatağında nitrik oksit'e duyarlılığın azalmasına ve yapısal veya indüklenebilir NOS aktivitesinin inhibe edilmesine bağlı olabileceği düşünüldü. Endotoksemi grubunda asetil koline verilen azalmış gevşeme cevaplarında birden fazla mekanizma ile gerçekleşmiş olabileceği öngörüldü. Aminoguanidin uygulaması endotoksemide azalmış vasküler gevşeme cevaplarını tamamen düzeltmiş ve bozulmuş vasküler cevabın aminoguanidin ile önlenebileceğini göstermiştir. LPS'in perfüzyon basıncında oluşturduğu akut ve konsantrasyona bağlı düşüşün perfüzyon ortamına eklenen L-NAME ile inhibe edilebilmesi de bu etkinin L-arjinin:NO yolağı üzerinden gerçekleştiğini gösterdi.

Anahtar kelimeler: Sepsis; Nitrik Oksit; L-arjinin:NO yolağı; izole böbrek perfüzyonu; NOS; Aminoguanidin

ROLE OF NITRIC OXIDE IN RAT KIDNEY VASCULAR BED IN ENDOTOXEMIA

Abstract:

Studies on sepsis's pathophysiology and treatment options have focused on cytokines and mediators. It was thought that the mediator, first called endothelium-derived relaxing factor and later determined to be nitric oxide (NO), may be necessary in this process. Our study aimed to investigate the changes in the renal vascular bed in endotoxemia and the effect of the L-arginine: NO pathway in this process.The study was carried out on isolated perfused kidney preparations from rats. While the control group was injected with intraperitoneal saline 4 hours before, the endotoxemia group was administered LPS (20mg/kg; IP).

No significant difference was detected in basal perfusion pressure and contractile responses to phenylephrine (0.1-3 μM) in the endotoxemia group. It was shown that endothelium-dependent relaxation responses to acetylcholine (0.1-1000 μg) were significantly reduced in the endotoxemia group. While aminoguanidine (20mg/kg; IP; 4 hours before) application did not change the endothelium-dependent relaxation responses to acetylcholine (0.1-1000 μg) in the control group, it completely corrected the decreased endothelium-dependent relaxation responses in the endotoxemia group and increased it to control levels. In the section aiming to determine the acute effect of LPS, LPS added to the perfusion solution did not change the basal perfusion pressure. After increasing the perfusion pressure with phenylephrine, LPS was shown to provide an acute, concentration-dependent, L-NAME (30 μM)-inhibited and non-repeatable decrease in perfusion pressure at concentrations of 3μg/ml and 10μg/ml.

It was thought that the lack of a significant difference in basal perfusion pressure and contractile responses to phenylephrine in the endotoxemia group may be due to decreased sensitivity to nitric oxide in the kidney microvascular bed secondary to increased nitric oxide production due to endotoxemia and inhibition of constitutive or inducible NOS activity. The decreased relaxation responses to acetylcholine in the endotoxemia group may have occurred through multiple mechanisms. Aminoguanidine application completely corrected the decreased vascular relaxation responses in endotoxemia and showed that the impaired vascular response could be prevented with aminoguanidine. The fact that the acute and concentration-dependent decrease in perfusion pressure caused by LPS could be inhibited by L-NAME added to the perfusion medium showed that this effect occurred through the L-arginine: NO pathway.

Keywords: Sepsis; Nitric Oxide; L-arginine, NO pathway; isolated renal perfusion; NOS; aminoguanidine

1. INTRODUCTION

Sepsis poses a serious problem in the health care settings with its increasing incidence and the high morbidity and mortality rate. Studies have shown that sepsis occurs with the host's response to infection rather than the direct effects of the disease. In this sense, it has been understood that the infection is the only factor that pulls the trigger and that the decisive factor is the cytokines and the mediators that cause the release of the cytokines (1). For this reason, studies on the pathophysiology and treatment options of sepsis have focused on cytokines and mediators. It was thought that the mediator, first called endothelium-derived relaxing factor and determined to be nitric oxide in 1987, may also be important in this process (2).

The primary determinant of mortality in sepsis is the progressive failure of the circulatory system and the resulting severe hypotension, decreased response to endogenous and exogenous agents, decreased vascular resistance, impaired flow regulation of organs and reduced oxygen supply to tissues (3). It has been determined that the source and target of a significant portion of the mediators effective in this response is the vascular endothelium. Nitric oxide (NO) is one of the most essential mediators (4). On the other hand, the vascular response of the kidney, one of the organs primarily affected by sepsis, and the factors influencing this response have not yet been fully explained.

In our study, we aimed to investigate the renal vascular bed response in perfused kidney preparations isolated from rats in which endotoxemia was induced by applying LPS and the effect of aminoguanidine, which is similar in structure to L-arginine and has been shown to selectively inhibit inducible nitric oxide synthase (iNOS), on this response. Thus, the aim was

to examine the role of the L-arginine: NO pathway in the response of the renal vascular bed in sepsis.

2. METHOD

The study was conducted on isolated perfused kidney preparations prepared from 74 male and female rats weighing 150-340 g in the Laboratory of Pharmacology Department of Hacettepe University Faculty of Pharmacy. In the first stage of the study, rats were divided into control and endotoxemia groups. By applying intraperitoneal (IP) physiological saline to the control group and IP lipopolysaccharide (LPS) to the endotoxemia group, the renal vascular bed response in endotoxemia and the role of the L-arginine: NO pathway in this response were evaluated. Additionally, the acute effect of LPS was assessed in isolated perfused kidneys prepared from 18 rats.

Preparation of isolated kidney preparations: Rats weighing 150-340 g were anaesthetized with urethane anesthesia (1.6 g/kg; IP). After entering the abdomen through a midline incision, the area where the left renal artery separates from the abdominal aorta was isolated by dissection. The abdominal aorta was turned and ligated just distal to the exit. The left renal artery was rotated and suspended. The abdominal aorta was returned and ligated proximal to the renal artery origin. An incision was made on the anterior aspect of the abdominal aorta at the origin of the renal artery. From here, the renal artery was catheterized with a perfusion cannula and identified by tying the suspended suture. To check that the kidney was perfused, Krebs-Henseleit Solution (KHS) was administered through the renal artery cannula before the preparation was removed, and 200 U/kg heparin was injected through the same route. Dissection separated the left kidney from the adrenal gland and surrounding tissues, and its capsule was peeled off. Finally, after cutting the abdominal aorta, renal vein and ureter, the preparation was placed in the perfusion system.

Isolated perfused kidney setup: The prepared, isolated kidney preparation was perfused with a peristaltic perfusion pump (Harvard Model 1203-a) with a KHS gassed with 95% O2 and 5% CO2 and kept constant at 37oC, with an average perfusion rate of 6 ml/min. Perfusion pressure was measured with the 'Pressure Transducer' (Ugo Basil Model 800) connected to the system with the Y leg and recorded on the printer (Ugo Basil Model 7070) through the

'Pressure Transducer Preamplifier' (Ugo Basil model 7082). Experiments were started after the isolated rat kidney was rested in the perfusion system for 40 minutes and the basal perfusion pressure stabilized (Figure 1).

Solutions and drugs used: Krebs-Henseleit Solution (mM): NaCl:95; KCL:4.7; $MgSO_4$:1.2; $CaCl_2$:2.5; KH_2AFTER_4:1.2; $NaHCO_3$:25; Glucose:11.1.

NaCl, $CaCl_2$, KCl, $MgSO_4$, KH_2PO_4, $NaHCO_3$ and urethane from Merck;α-D(+)-Glucose, N^G-nitro-L-arginine methyl ester (L-NAME), lipopolysaccharide (*E.coli* serotype 055:85), papaverine, phenylephrine, indomethacin, acetylcholine and aminoguanidine were provided by Sigma.

Subject groups and vascular responses examined:

Control and endotoxemia groups: In the study, rats (n = 56) were divided into endotoxemia and control groups. Four hours beforehand, LPS was administered IP at a dose of 20mg/kg in physiological saline to the rats (n=22) that would be subjected to endotoxemia. Rats in the control group (n=34) were given only physiological saline, IP. In these groups, the contraction response of the renal vascular bed and the endothelium-dependent relaxation responses were evaluated.

Contractile response of the renal vascular bed: Phenylephrine (0.1-3μM) was added to the perfusion medium after the perfusion pressure stabilized in the endotoxemia group (n = 8) and the control group (n = 12). Concentration-dependent contraction responses were determined, and concentration-response curves were obtained.

Endothelium-dependent relaxation response of the renal vascular bed: The endothelium-dependent relaxation response of the renal vascular bed was examined in rats with endotoxemia (n = 14) and the control group (n = 18). The effect of aminoguanidine (20mg/kg; IP; 4 hours before) was investigated in 6 rats in the endotoxemia group and 5 in the control group. Kidney preparations isolated from rats in all four groups were perfused (6 ml/min) with KHS, to which indomethacin (10μM) was added. Perfusion pressure was increased by adding phenylephrine (0.1-3μM) to the perfusion solution at a concentration that achieved 70-80% of the maximum phenylephrine contraction in the renal vascular bed. Endothelium-dependent relaxation responses were obtained by administering acetylcholine (0.1-1000μg) to

the perfusion system as bolus injections from the proximal part of the isolated kidney preparation. In preliminary studies, it was found that the dose of papaverine that provided the most endothelium-independent vascular smooth muscle relaxation was 0.1 mg. After obtaining maximum relaxation response with acetylcholine, a bolus papaverine 0.1 mg injection was made.

In the kidney preparation isolated from another group of rats (n=4), endothelium-dependent relaxation responses to acetylcholine were evaluated in the presence of the L-arginine analogue, NG-nitro-L-arginine methyl ester (L-NAME) (30μM) added to the perfusion solution.

To express the endothelium-dependent relaxation responses in terms of the maximum relaxation capacity of the renal vascular bed, the relaxations provided by acetylcholine were calculated as the percentage of papaverine relaxation, and concentration-response curves were obtained.

The acute effect of lipopolysaccharides: To evaluate the acute effect of LPS on the renal vascular bed, perfused kidney preparations were isolated from rats (n = 18) that did not receive any treatment. The impact of LPS added to the perfusion solution at 3-10 μg/ml concentrations on basal perfusion pressure was investigated. In another group of preparations, the effect of LPS at concentrations of 3 μg/ml (n=8) and 10 μg/ml (n=6) added to the perfusion solution after increasing the perfusion pressure with phenylephrine was examined. The effect of 3 μg/ml LPS on perfusion pressure was also evaluated in the presence of L-NAME (30 μM) (n=4).

Statistical evaluation: All values in the text and figures are the average of n observations ± expressed as standard error. The non-parametric Mann-Whitney U test was used to compare groups. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

In this study, carried out with isolated rat kidney perfusion, the targeted results were obtained using three main methods. The first is the phenylephrine (0.1-3μM) contractile response of the renal vascular bed; the second is the endothelium-dependent relaxation response caused

by acetylcholine (0.1-1000μg); and the third is the acute response caused by LPS (3-10 μg/ml) perfusion.

Basal perfusion pressure:

No statistically significant difference was detected between the basal perfusion pressures detected in isolated perfused kidney preparations prepared from the control (n = 34) and LPS (n = 22) groups (Figure 2).

Contractile response of the renal vascular bed:

In the control group (n=12), phenylephrine (0.1-3μM) added to the perfusion medium increased the perfusion pressure in a concentration-dependent manner (Table 1). When both groups were compared, no significant difference was detected in the contraction response of phenylephrine at any concentration ($p>0.05$) (Figure 3).

Endothelium-dependent relaxation response of the renal vascular bed:

In the control group (n=13), acetylcholine (0.1-1000μg) decreased perfusion pressure dose-dependently (Table 2). L-NAME (30 μM) added to the perfusion solution significantly reduced the relaxation response of acetylcholine ($p<0.05$) (Figure 4).

The relaxation response obtained with acetylcholine (0.1-1000μg) in the LPS group (n=8) was found to be significantly less than the control group at all doses ($p<0.05$) (Table 2; Figure 5).

In the aminoguanidine-administered group (n=5), acetylcholine (0.1-1000μg) decreased perfusion pressure in a dose-dependent manner (Table 2). Compared to the control group, it was determined that aminoguanidine application did not change the relaxation response caused by acetylcholine at any dose ($p>0.05$) (Figure 6).

When aminoguanidine was administered together with LPS (n=6), it was determined that acetylcholine (0.1-1000μg) decreased the perfusion pressure dose-dependently (Table 2), and this application significantly improved the acetylcholine response at all doses compared to the LPS-only group ($p<0.05$) (Figure 6). No significant difference was detected between the responses obtained from the groups administered aminoguanidine together with LPS ($p>0.05$) (Figure 6).

Acute effect of LPS perfusion:

Adding 3 and 10 µg/ml LPS to the perfusion solution did not significantly affect the basal perfusion pressure in perfused kidney preparations isolated from rats without any treatment ($p>0.05$). On the other hand, after increasing the perfusion pressure with phenylephrine, the application of LPS at concentrations of 3 µg/ml (n=8) and 10 µg/ml (n=6) decreased the perfusion pressure in a concentration-dependent manner (Figure 7). Adding L-NAME (30 µM) to the perfusate significantly reduced the vasodilator effect obtained with 3 µg/ml LPS perfusion ($p<0.05$) (Figure 7).

4. DISCUSSION

The kidney is one of the organs most primarily affected by sepsis. In the study, changes in the kidney vascular bed were investigated in the endotoxemia model created in rats. The contraction responses to phenylephrine and relaxation to acetylcholine, the role of the L-arginine: NO pathway in this process, and the effect of aminoguanidine, a selective iNOS inhibitor, were tried to be evaluated.

Our study detected no significant difference between the basal perfusion pressures in isolated perfused rat kidneys prepared from the control and endotoxemia groups. However, different results may be obtained in different endotoxemia models.

Phenylephrine added to the perfusion medium in isolated perfused rat kidneys was 20 mg/kg in the control group and 4 hours before. In the endotoxemia group administered IP LPS, perfusion pressure increased concentration independently, but no significant difference was detected in this response at any concentration. Julou-Schaffer et al. showed that the contractile response to norepinephrine decreased in the aortic rings isolated from rats, in which they induced endotoxemia by applying IP LPS 20 mg/kg 4 hours before. They found that LPS infusion (5 mg/kg/hour; iv) reduced the contractile response to norepinephrine under in vitro conditions, and they showed that L-NMMA strengthened this response in both conditions (5). Similarly, in groups made endotoxemia by applying LPS KCL, norepinephrine and 5-hydroxytryptamine were increased in rat aortic rings (6) to norepinephrine in rat femoral artery (7), to thromboxane mimetic U46619 in an isolated perfused heart preparation in rabbits (8). It was shown that there was a significant decrease in the contractile responses compared to the control groups, and it was determined that L-arginine analogues could

reverse these responses. In a study on rats, Szabo et al. showed that the administration of LPS (10 mg/kg; iv) caused a significant decrease in the contractile response to norepinephrine 30 and 60 minutes after LPS administration in an in vivo setting (9). These results indicate that there is a decreased response to contractile agents in endotoxemia and increased NO production is the primary mechanism. On the other hand, in our study, no decrease was detected in the contraction response caused by phenylephrine in the endotoxemia group in the isolated perfused rat kidney compared to the control. An increasing response was even obtained, but the difference was statistically insignificant. The differences in the results from other studies may be due to the different vascular beds examined. There may be a difference between a macrovascular structure such as the aorta and an isolated perfused kidney microvascular bed. The difference in phenylephrine responses in the control and endotoxemia groups may be secondary to a decrease in the sensitivity of the renal vascular bed to NO secondary to increase NO formation due to endotoxemia. This effect may be explained by increased NO production or inhibition of constitutive (10) and/or inducible (11) NOS activity by LPS. The balance established between the decrease in NO release from the endothelium secondary to the damage to the endothelium in endotoxemia and the inducible NOS activity and the excess NO released from macrophages, neutrophils and vascular smooth muscle can ensure that contractile responses do not change in endotoxemia.

In isolated perfused rat kidneys, acetylcholine dose-dependently reduced perfusion pressure. The fact that the NOS inhibitor L-NAME added to the perfusion solution reduces the relaxation response of acetylcholine shows that this response in the kidney occurs through NO. It was determined that the relaxation response obtained with acetylcholine in the endotoxemia group was significantly less than the control group at all doses. In a study on dogs, Wylam et al. showed that the endothelium-dependent relaxation responses to acetylcholine in the femoral, renal and mesenteric vessels did not change in the control and endotoxemia (5 mg/kg; iv; 4 hours before) groups, whereas the endothelium-dependent relaxation responses to acetylcholine decreased significantly in the endotoxemia group (12). When the decrease in the endothelium-dependent relaxation response to acetylcholine in the endotoxemia group was evaluated in our study, it was predicted that more than one factor might contribute to this result. It may be due to the inability of NO to affect the vascular smooth muscle due to physical obstacles, such as oedema in the vascular wall during the endotoxemia process and the inability of the vascular smooth muscle to respond to NO. Apart

from this, there is a decrease in the muscarinic receptor population affected by acetylcholine in endotoxemia (13), or the elements that provide communication between the receptors in the endothelium and NOS may also be affected. NO, whose synthesis increases with the induction of iNOS in the vascular bed in endotoxemia, may cause 'down-regulation' of the structural NOS isoform in the endothelium. LPS can inhibit the structural NOS isoform with its direct effect on vascular endothelium (14). A decrease in sensitivity to NO may occur secondary to increase NO formation in resistant vessels. In endotoxemia, the amount of L-arginine in the environment may be the rate-determining step, and NO synthesis in the endothelium may decrease due to substrate deficiency (5,7) NO can be inactivated by oxygen-dependent free radicals formed in activated neutrophils. NO may cause endothelial cell damage due to its cytotoxic effects. In addition, NO has both structural (10) and can also be induced (11). It has been shown that it can irreversibly inhibit NOS. Additionally, activation of endothelial cells with cytokines or endotoxin causes endothelial cell death (15).

In our study, endothelium-dependent relaxation responses to acetylcholine were obtained in the isolated perfused rat kidney in the group administered aminoguanidine, a NOS inhibitor. It was determined that aminoguanidine application did not affect this response compared to the control group. When aminoguanidine was administered together with LPS, it was observed that it significantly preserved the decrease in endothelium-dependent relaxation responses to acetylcholine at all doses compared to the LPS-only group. In addition, the response obtained in the group administered aminoguanidine with LPS did not differ significantly from the control and aminoguanidine-only groups. This effect shows that the aminoguanidine application preserves the relaxation response due to the damaged endothelium we detected during the endotoxemia process. Aminoguanidine, which came to the fore with its effectiveness in preventing diabetes-related complications, was later shown to inhibit NO production (16). A study conducted on non-diabetic rats to evaluate the effects of aminoguanidine on structural NOS revealed that aminoguanidine and L-NMMA applications increased the average blood pressure in a dose-dependent manner, but L-NMMA was 40 times more effective. This finding shows that although the effectiveness of aminoguanidine and L-NMMA in iNOS inhibition is the same, L-NMMA is 40 times more effective in constitutive NOS inhibition (16). Wu et al. found a significant decrease in mean blood pressure and contractile response to norepinephrine 180 minutes after LPS administration in rats. In rats administered aminoguanidine (15 mg/kg; iv) 20 minutes before LPS, the average

blood pressure remained significantly higher, and in addition, the average blood pressure increased in rats administered aminoguanidine (15 mg/kg and 45 mg/kg) in increasing doses 180 minutes after LPS. It increased blood pressure dose-dependently and reversed hypotension. The contraction response caused by phenylephrine returned to normal in LPS rats treated with aminoguanidine (17).

In light of this information, the fact that aminoguanidine application did not significantly change the relaxation response to acetylcholine compared to the control in our study shows that in vivo application does not affect structural NOS in the isolated perfused renal vascular bed. On the other hand, when administered with LPS, it completely prevented the decrease in the relaxation response to acetylcholine detected in the endotoxemia group. This effect prevented the deterioration in the endothelium-dependent relaxation response, which is detected and essential in the kidney's endotoxemia process.

Then, the acute effect of LPS added to the perfusion solution on the renal vascular bed was evaluated. LPS perfusion did not change basal perfusion pressure in the isolated rat kidney. In contrast, it produced a significant concentration-dependent decrease in perfusion pressure after perfusion pressure was increased with phenylephrine. Baydoun et al. determined that LPS (0.1-100 μg/ml) perfusion caused potent vasodilation in the isolated perfused rat heart after increasing the basal perfusion pressure with U46619 (18). In our study, L-NAME significantly reduced the vasodilator effect obtained by LPS perfusion, indicating that this effect is through the L-arginine: NO pathway. However, it is thought that this acute effect, which is related to NO synthesis and concentration-dependent, is not secondary to LPS increasing inducible NOS activity because Szabo et al. detected a moderate iNOS activity only in the rat aorta 60 minutes after LPS (10 mg/kg; iv; bolus) application, and they showed increased iNOS activity in tissues such as lung, liver, mesentery and heart only after 180 minutes (9). iNOS activity reaches its maximum only at the 6^{th} hour. It decreases to the basal level at the 24^{th} hour (9), and we can say that LPS provides its acute effect in the kidney by increasing structural NOS activity.

5. CONCLUSION

As a result, in the endotoxemia model created in rats with LPS application, it was shown that although the basal kidney perfusion pressure and the response to contracting agents were

preserved, the endothelium-dependent relaxation response via the L-arginine: NO pathway was impaired, and it was determined that this could be prevented by aminoguanidine application. In addition, it has been determined that LPS causes acute vasodilation in the renal vascular bed via the L-arginine: NO pathway, with its acute effect. However, further studies are needed to fully elucidate these results' mechanisms and determine the protective effect of aminoguanidine on vascular responses affected in sepsis.

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Figure 1: Experimental setup used in isolated perfused rat kidney.

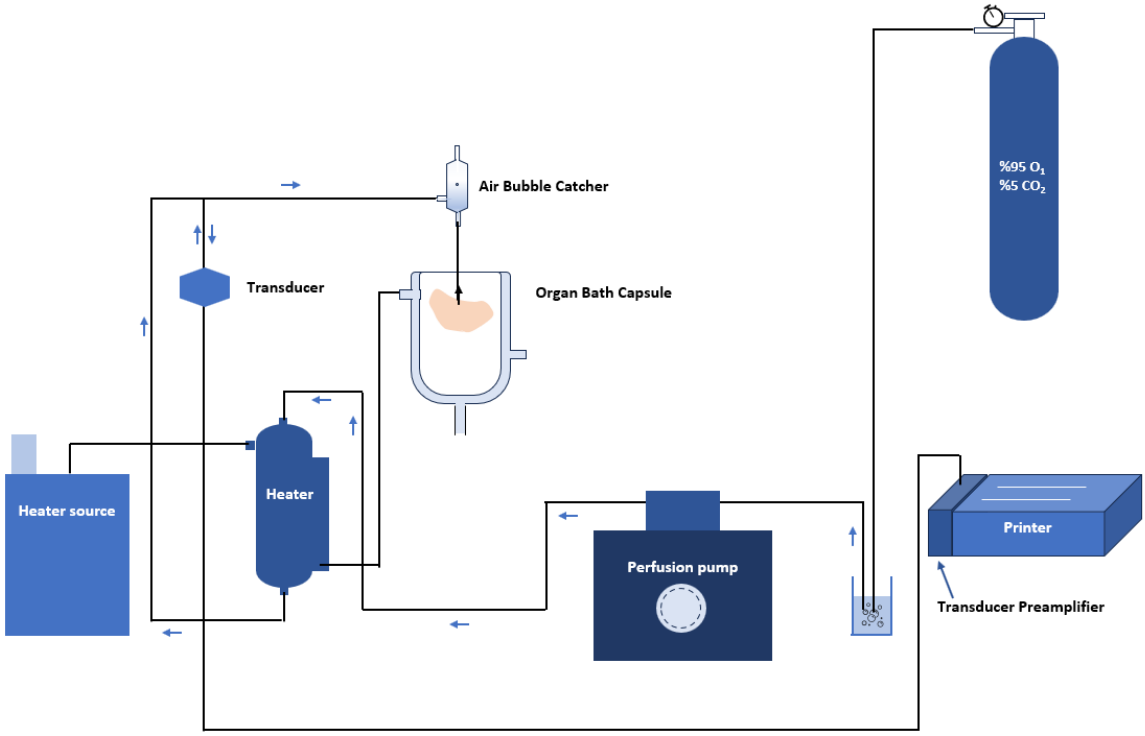

Figure 2: Basal perfusion pressure values in kidneys isolated from control (n=34) and LPS (n=22) group rats. Values are expressed in mmHg and given as mean ± standard error.

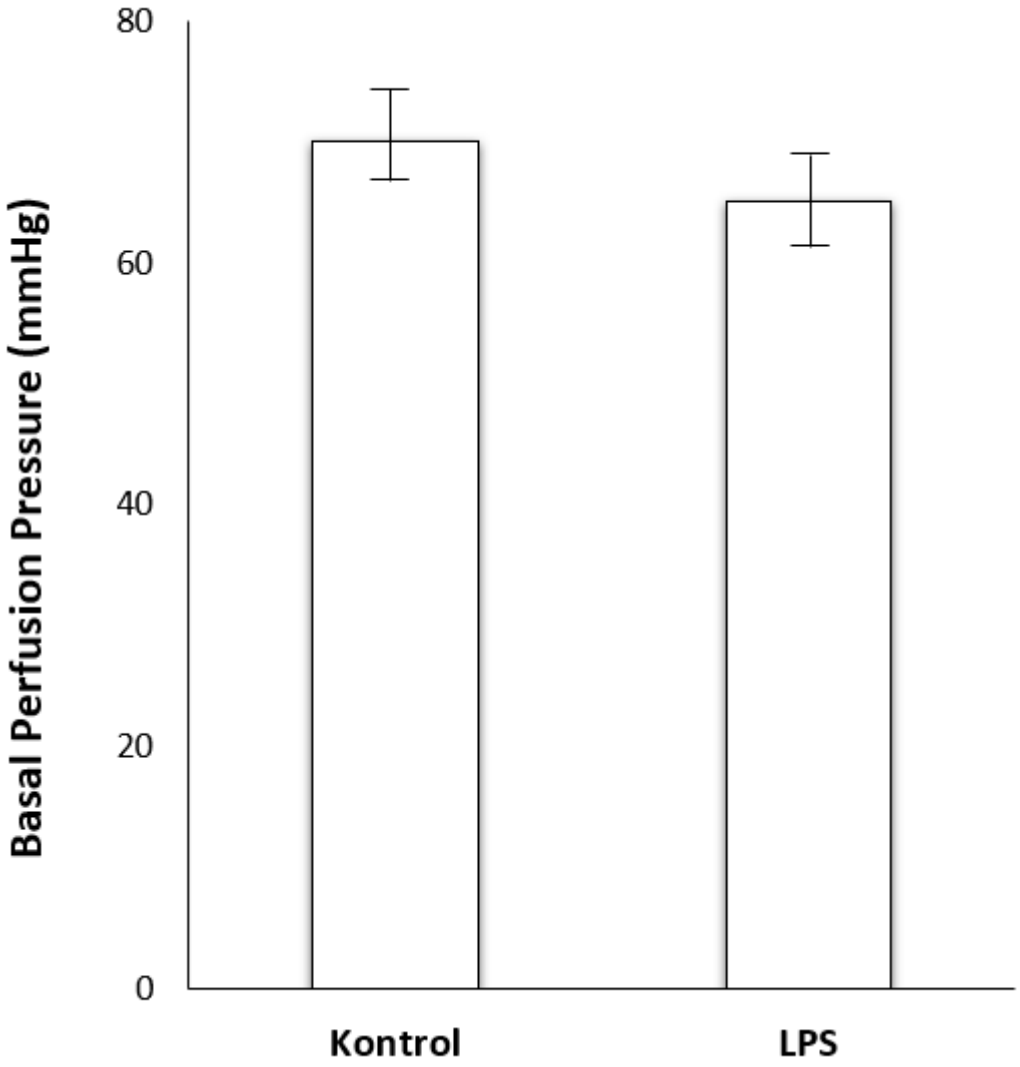

Figure 3: Concentration-response curves of the increase in perfusion pressures obtained with phenylephrine in the isolated perfused rat kidney in control (□) (n = 12) and LPS (■) (n = 8) groups. Values are expressed in mmHg and given as mean ± standard error.

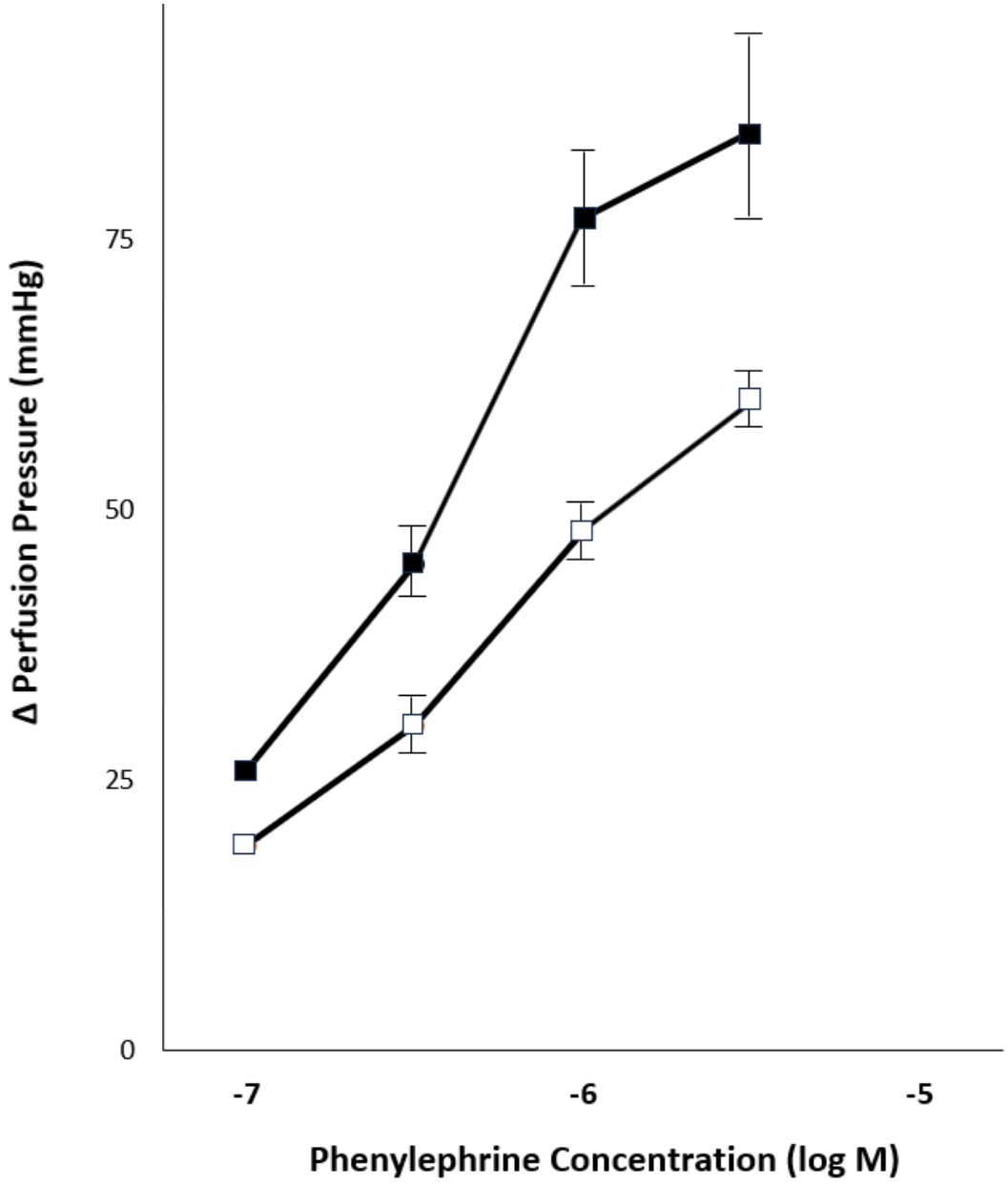

Figure 4: Concentration-response curves of endothelium-dependent relaxation responses obtained with acetylcholine in the isolated perfused rat kidney in the control group (□) and in the presence of 30 µM L-NAME in the control group (■). Values are expressed as the % of papaverine relaxation, and the mean ± the standard error. (*Statistically different from the relaxation response obtained in the control group (□) p<0.05.)

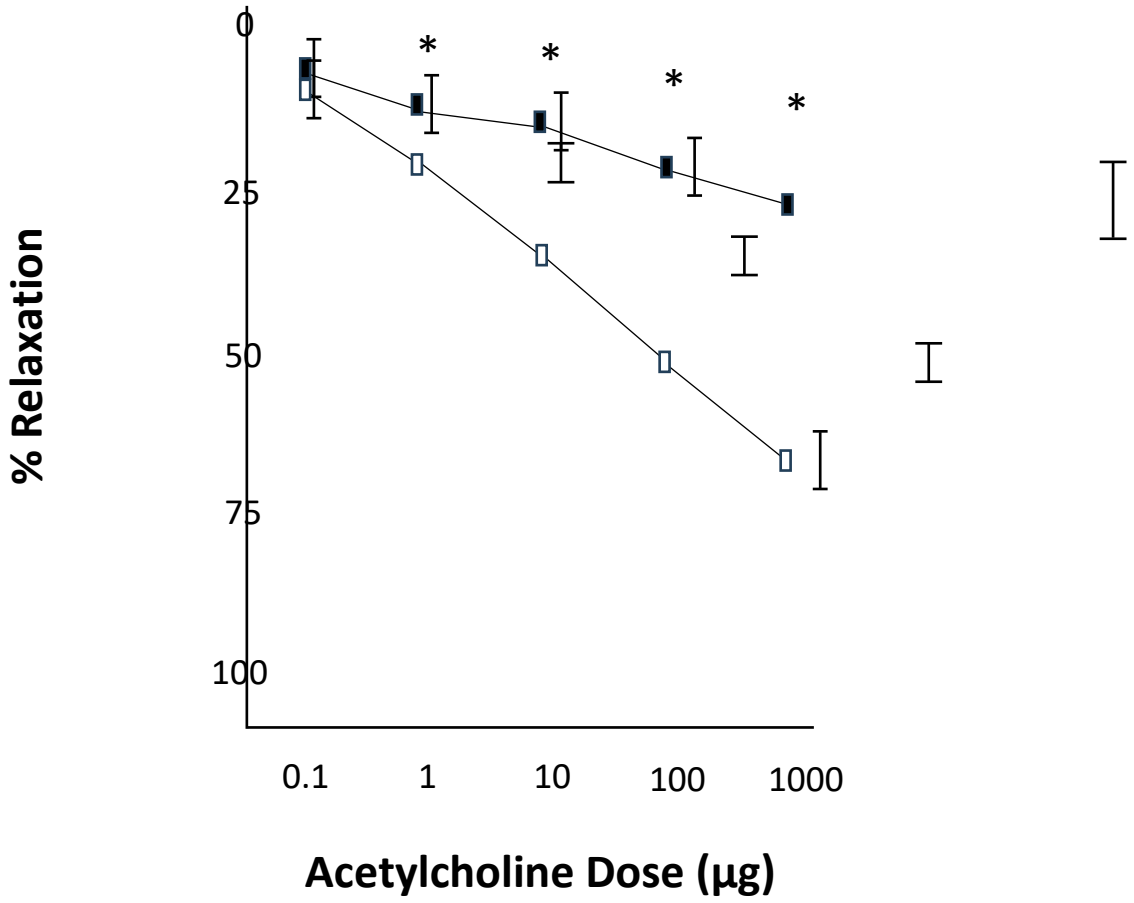

Figure 5: Concentration-response curves of endothelium-dependent relaxation responses obtained with acetylcholine in isolated perfused rat kidneys in control (□) and LPS (■) groups. Values are expressed as % of papaverine relaxation and are given as mean ± standard error.

(*Statistically different from the relaxation response obtained in the control group (□) $p<0.05$.)

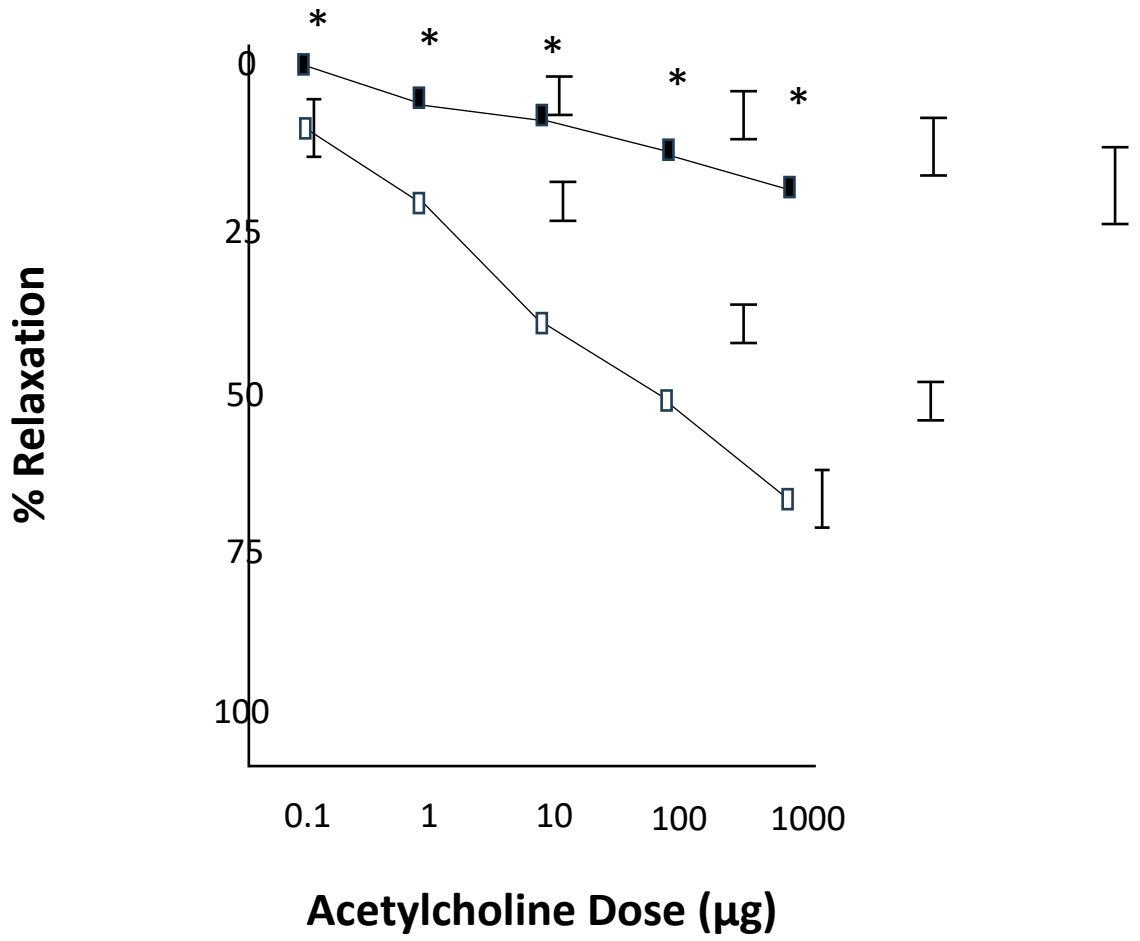

Figure 6: Concentration-response curves of endothelium-dependent relaxation responses to acetylcholine in perfused rat kidneys isolated from control (□) and LPS (■) groups and aminoguanidine-applied control (▽) and LPS (▼) groups. Values are expressed as % of papaverine relaxation and are given as mean ± standard error. (*Statistically different from the relaxation response obtained in the LPS group (■) p<0.05.)

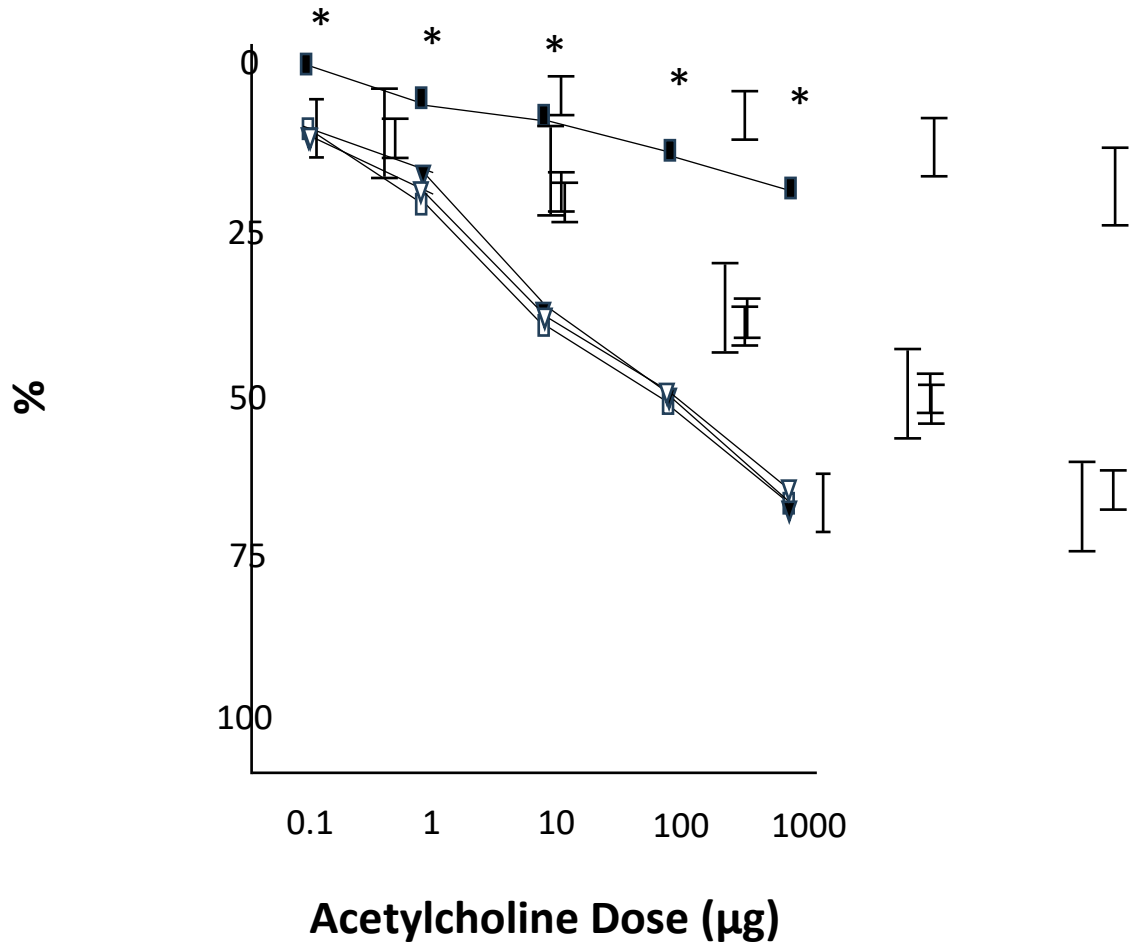

Figure 7: Relaxation responses obtained with 3 µg/ml and 10 µg/ml LPS perfusion in the isolated perfused rat kidney and with 3 µg/ml LPS perfusion in the presence of 30 µM L-NAME. Values are expressed as a percentage of phenylephrine contraction, and the mean is given as ± standard error.

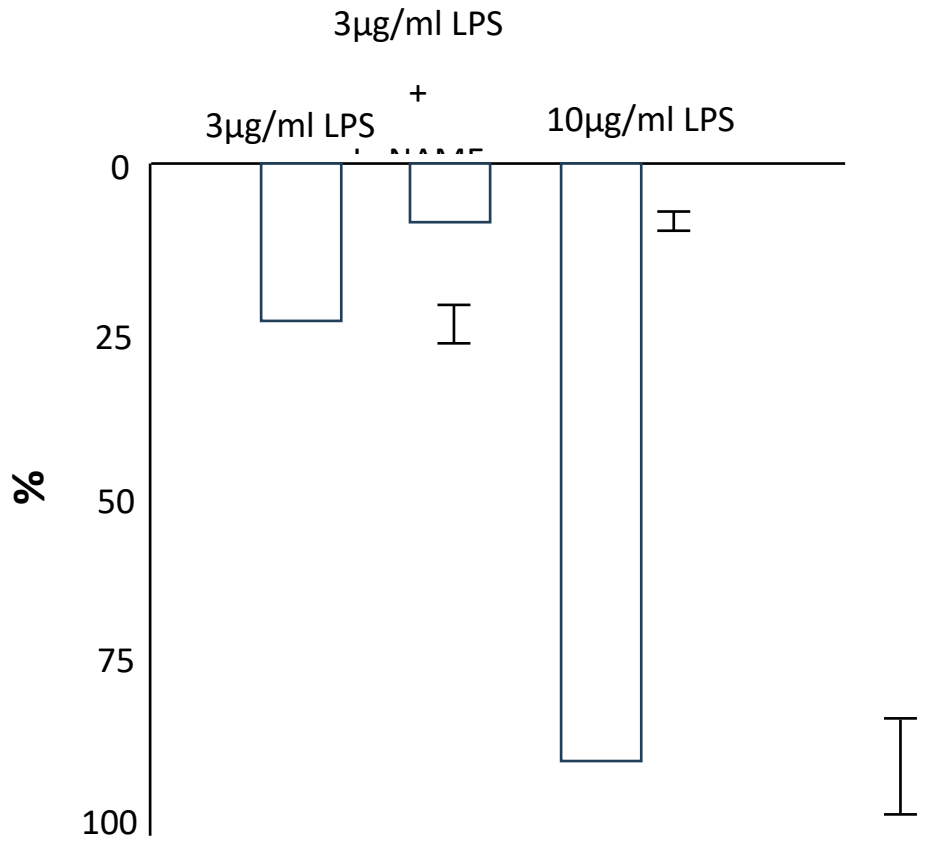

Table 1: Increase in perfusion pressure achieved with phenylephrine in isolated perfused rat kidney. Values are expressed in mmHg and given as mean ± standard error (n=number of subjects).

	n	0.1µM	0.3µM	1µM	3µM
CONTROL	**12**	**17±2.6**	**33±3.5**	**52.25±3.6**	**65.25±5.5**
LPS	**8**	**28.1±1.1**	**40.5±9.0**	**68.6±8.7**	**82.5±10.9**

Table 2: Endothelium-dependent relaxation responses to acetylcholine in isolated perfused rat kidney. Values are expressed as % of papaverine relaxation and are given as mean ± standard error (n=number of subjects).

	n	0.1µg	1µg	10µg	100µg	1mg
CONTROL	**13**	**12.3 ±1.7**	**23.2 ±1.9**	**41.2 ±2.7**	**58.1 ±2.9**	**68,8 ±3.3**
CONTOL / AMINOGUANIDINE	**5**	**12.3 ±1.7**	**21.8 ±3.7**	**37.3 ±2.5**	**55.3 ±3.4**	**65.3 ±5.0**
LPS	**8**	**3.2 ±1.4**	**6.8 ±1.9**	**13.1 ±2.7**	**19.1 ±3.8**	**24.7 ±3.7**
LPS AMINOGUANIDINE	**6**	**13.1 ±2.6**	**20.2 ±3.6**	**35.3 ±6.1**	**51.6 ±6.9**	**67.5 ±4.5**