Revista de Ciências Farmacêuticas Básica e Aplicada
*Journal of Basic and Applied Pharmaceutical Sciences*

**Rev. Ciênc. Farm. Básica Apl.,** v. 28, n.3, p. 341 - 345, 2007
ISSN 1808-4532

# Vascular hyporeactivity to angiotensin II induced by Escherichia coli endotoxin is reversed by Nω-Nitro-L-Arginine, an inhibitor of nitric oxide synthase

Rodrigues, L.A.[1]*; Fracasso, J.F.[2]

*[1]Faculdade de Farmácia-Bioquímica, Faculdades Unificadas da Fundação Educacional de Barretos, Barretos, SP, Brasil.*
*[2]Laboratório de Farmacologia, Departamento de Princípios Ativos Naturais e Toxicologia, Faculdade de Ciências Farmacêuticas, UNESP, Universidade Estadual Paulista, Araraquara, SP, Brasil.*

Recebido 03/10/2007 / Aceito 28/04/2008

## ABSTRACT

**Septic shock or sepsis is reported to be one of the major causes of death when followed by systemic infectious trauma in humans and other mammals. Its development leads to a large drop in blood pressure and a reduction in vascular responsiveness to physiological vasoconstrictors which, if not contained, can lead to death. It is proposed that this vascular response is due to the action of bacterial cell wall products released into the bloodstream by the vascular endothelium and is considered a normal response of the body`s defenses against infection. A reduction in vascular reactivity to epinephrine and norepinephrine is observed under these conditions. In the present study in rats, the aim was to assess whether those effects of hypotension and hyporeactivity are also related to another endogenous vasoconstrictor, angiotensin II (AII). We evaluated the variation in the power of this vasoconstrictor over the mean arterial pressure in anesthetized rats, before and after the establishment of hypotension by *Escherichia coli* endotoxin (Etx). Our results show that in this model of septic shock, there is a reduction in vascular reactivity to AII and this reduction can be reversed by the inhibitor of nitric oxide synthase, Nω-Nitro-L-Arginine (NωNLA). Our results also suggest that other endogenous factors (not yet fully known) are involved in the protection of rats against septic shock, in addition to the L-arginine NO pathway.**

*Keywords:* vascular hyporeactivity; NO; rat; angiotensin II; NωNLA *Escherichia coli* endotoxin.

## INTRODUCTION

Septic shock, a trauma following an infectious process, is a major cause of death in the human population (Titheradge, 1999; Ambrósio & Fracasso, 2000). Acute inflammation causes endothelial dysfunction, which is partly mediated by oxidative stress and activation of nitric oxide (Mittermayer et al., 2005). Death occurs by circulatory collapse and development of systemic dysfunction of vital organs, including other clinical conditions such as acute lung injury shock, renal failure, and multiple organ failure (Ambrósio & Fracasso, 2000; Fracasso et al., 2003). These conditions are critical when the pathogens are Gram-negative bacteria such as *Escherichia coli*, which release endotoxin (Etx) that provokes an acute release of powerful endogenous mediators into the blood (Thiemermann & Vane, 1990; Fracasso et al., 1996). There are no records in the literature of similar events occasioned by toxins from other microorganisms.

Etx is a lipopolysaccharide component of the Gram-negative bacterial wall that is responsible for many of the pathophysiological events that occur during septic shock (sepsis). It is suggested that Etx-induced sepsis in rats is due to the release of several cytokines that in turn induce the release of other cytokines (interleukin-1, interleukin-6, interferon-γ) and the tumor necrosis factor-α (TNF-α ), which act as an amplifying mechanism in the inflammatory response (Bone et al., 1992; Ferreira et al., 1993). Despite advances in treatment, sepsis is still associated with high mortality (Pleiner et al., 2003). TNF-α plays an important role in Etx-induced hyperalgesia and its presence is essential for the release of other cytokines. On the other hand, vascular hyporeactivity to catecholamine is one of the main features

*Autor Correspondente:* Luiz Alves Rodrigues - Faculdade de Farmácia-Bioquímica - Faculdades Unificadas da Fundação Educacional de Barretos - Av. Prof. Roberto Frade Monte, 389 - Bairro Aeroporto - CEP: 14.783-266 - Barretos - SP, Brasil - Email : alveslar@ig.com.br.

of sepsis and Etx reproduces this hyporeactivity in both animals and humans (Ambrósio & Fracasso, 2000; Pleiner et al., 2003). Etx also activates the endothelial constitutive nitric oxide synthase (cNOS), with consequent overproduction of nitric oxide (NO) that results in reduced contractile responsiveness to norepinephrine and an acute fall in blood pressure. In addition, an inducible isoform of NOS (iNOS) is induced in various organs and in the blood vessel walls, further decreasing the vascular reactivity *in vivo* and reducing the blood pressure in rats and patients with sepsis (Pleiner et al., 2003). While the increase of bacterial Etx-induced NO in rats plays an important protective action in against the infection itself (Pleiner et al., 2003), it can lead to death of the rat if excessive. Systemic and regional vascular hyporeactivity to adrenoceptor agonists has been demonstrated following the administration of low doses of Etx to humans (Yoshikawa 1998). This hyporeactivity was detected together with hypotension, in the presence of increased levels of nitric oxide (NO) in the blood.

Etx can nonspecifically influence the course of an infection. The increase or decrease of its pathogenicity depends on the dose and administration route and the time elapsed between doses. An increase in the host resistance is observed when small doses of Etx are given 24 hours before an experimental infection or a lethal dose of Etx. Conversely, if Etx is given immediately before the pathogen, the animals become more susceptible to infection or lethal doses of Etx (Ferreira et al., 1993; Klosterhalfen & Bhardwaj, 1998).

The mechanisms by which Etx enhances host resistance can be separated into two phases: an early, nonspecific phase, clearly distinguishable from a later, antibody-dependent phase. The latter involves distinct humoral and cellular interactions, of which activation of the mononuclear-phagocytic system with subsequent effective clearance of infective agents from the host seems to be most prominent. On the other hand, the interaction of Etx with cells of higher organisms (lymphocytes, macrophages, mast cells, etc) results in the production and release of biologically active substances, such as bradykinin, serotonin and prostaglandins, which mediate a number of immunological reactions (Flohé et al., 1991). These mediators play a part in inflammation, fever induction, cell proliferation, differentiation and other biological phenomena (Thiemermann & Vane, 1990).

It has been suggested that the Etx-induced NO overproduction contributes to the acute low resistance to treatment for low blood pressure and hyporeactivity to endogenous and exogenous catecholamines and vasopressin, in the multiple organ dysfunction syndrome observed in sepsis. A conceivable pharmacological approach to protect tissues against the deleterious effects of excessive NO production includes specific inhibition of iNOS by Nω-Nitro-L-Arginina (NωNLA).

The current experiments were performed to characterize the protective effects of NωNLA against Etx-induced decreased vascular reactivity to Angiotensin II (AII) *in vivo* (Nava et al., 1991; Thiemermann & Vane, 1990).

## MATERIALS AND METHODS

### Endotoxin and Drugs

The Etx from *Escherichia coli* serotype 026:B6 was from Difco, Angiotensin II from Sigma Chemical Co. (St. Louis, USA), NωNLA from RBI laboratories and thiopental sodium from Cristália Laboratories (Itapira, SP, Brazil).

Drug solutions: AII, Etx, NωNLA were dissolved in saline (0.15M NaCl solution).

### Animals

The male Wistar rats (220-300g) were from the Department of Active Principles and Toxicology of the School of Pharmaceutical Sciences, Unesp at Araraquara (SP, Brazil). They were maintained under controlled conditions (22 ± 1°C; relative humidity 55 ± 5 %; 12/12 h dark/light cycle) with free access to water and rat feed.

### Methods

The rats were anaesthetized with thiopental sodium (40 mg/kg b.w. intraperitoneal). The experiments were carried out during daylight hours.

The trachea was cannulated to facilitate respiration. The right carotid artery was cannulated and connected to a pressure transducer (ANAMED 1000), which was connected to a physiograph to record the variations in mean arterial blood pressure (MAP). A cannula was also placed in the left jugular vein for the administration of drugs.

After being connected to the physiograph, the rat was allowed to stabilize for 30 min. Pressor responses were recorded after successive intravenous (i.v) injections of 0.5 and 1.0 μg/kg of AII at intervals of 2 min. To observe the effects of Etx (5.0 mg/kg) and NωNLA (25 mg/kg) on AII-induced increases in MAP, the pressor responses to AII were again assayed 1 hour after Etx, NωNLA or saline were injected (i.v.). NωNLA was injected 60 min after the Etx-induced hypotension had been observed.

### Statistical Analysis

The results were expressed as mean + standard error of the mean (SEM) of n experiments. They were compared by analysis of variance (ANOVA), followed by the Bonferroni Test. When $P < 0.01$, the difference was considered significant (Winner et al., 1991).

## RESULTS

### Demonstration chart

The mean arterial pressure (MAP) was increased by AII (0.5 and 1.0 μg/kg i.v.) in a dose-dependent way in control rats (Figure 1).

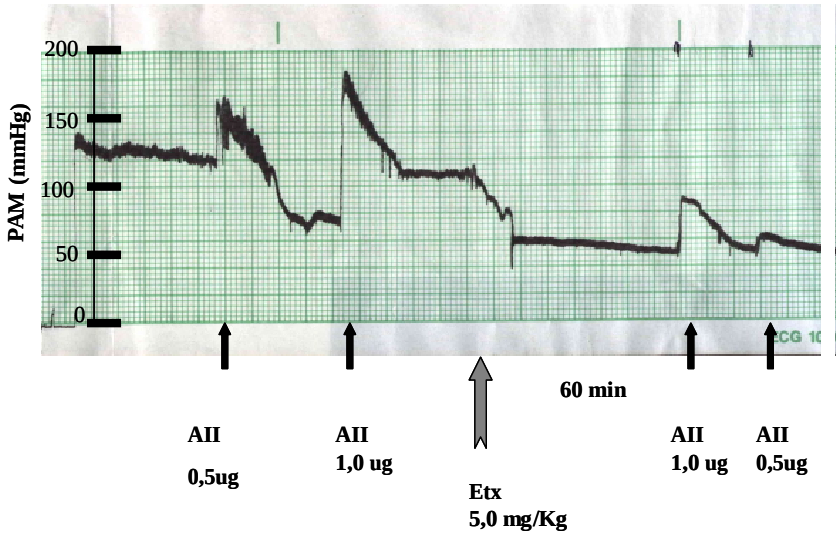


Figure 1. Representative chart recording (single animal) of mean arterial pressure (MAP) alterations induced by AII (0.5 and 1.0 µg/kg i.v.), recorded before (control) and 60 min after treatment with Etx (5.0 mg/kg i.v.).

**Effect of Etx**

The MAP was reduced significantly 60 min after Etx (5.0 mg/kg i.v.), as can be observed in Figure 2.

Control: basal 118.8 ± 2.5, AII (0.5 µg/kg i.v.) 149.7 ± 74.9 and AII (1.0 µg/kg i.v.) 177.5 ± 4.7; after Etx (5.0 mg/kg): basal 71.5 ± 6.6, AII (0.5 µg/kg i.v.) 88.2 ± 4.5, AII (1.0 ?g/kg i.v.) 99.0 ± 5.2 [mm of Hg].

**Effect of NωNLA**

The effect of Etx on MAP was reversed 60 min. after the injection of NωNLA (25 mg/kg i.v., 60 min after effects of Etx were observed), as can be seen in Figure 3. Control: basal 121.3 ± 1.8, AII (0.5 µg/kg i.v.) 147.5 ± 2.0, AII (1.0 µg/kg i.v.) 179.2 ± 4.5; after Etx (5.0 mg/kg): basal 71.3 ± 1.8 *, AII (0.5 µg/kg i.v.) 86.7 ± 1.2 *, AII (1.0 µg/kg

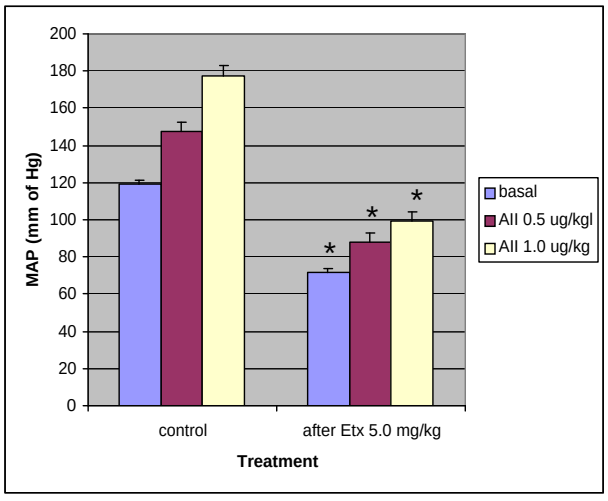


Figure 2. Mean arterial pressure (MAP). Responses to AII (0.5 and 1.0 µg/kg i.v) were recorded before (control) and 60 min. after Etx injection (5 mg/kg i.v.) in rats.
* P < 0.01 (n = 6), in relation to control.

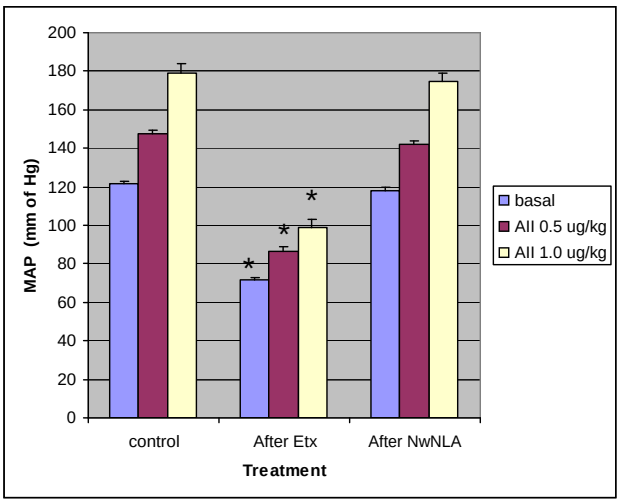


Figure 3. Mean arterial pressure (MAP). Pressor responses induced by AII (0.5 and 1.0 µg/kg i.v. ) recorded before (control), after 60 min. of the treatment with ETX ( 5.0 mg/kg i.v.) and 60 min. after the administration of NωNLA (25 mg/kg i.v.) in rats.
* P < 0.01 (n=6), in relation to control.

i.v.) 98.7 ± 2.7*, and after NωNLA: basal 117.7 ± 4.5, AII (0.5 μg/kg i.v.) 141.8 ± 4.1, AII (1.0 μg/kg i.v) 174.5 ± 4.3 [mm of Hg).

Pressor responses to AII were unaltered by administration of saline, compared to Etx (data not shown).

## DISCUSSION

Sepsis is characterized by signs and symptoms of decreased vascular reactivity, in parallel with hypotension resulting from the intense secretion of relaxing factors essentially by blood-vessel endothelial cells, in response to substances released from the cell walls of infecting bacteria. This process can progress to death. The vascular reactivity to catecholamines (epinephrine and norepinephrine) is seen to diminish in Etx-induced hypotension, but there are no published data on the vasoconstrictor effect of angiotensin II under these conditions. Studies have shown that the vasoconstrictor potency of norepinephrine is significantly reduced after pretreatment of the animal with Etx (LPS from *Escherichia coli*) and that this can be reversed by an inhibitor of cNOS, NωNLA (Ambrosio & Fracasso, 2000).

The hypotension during Etx-induced sepsis may reflect increased synthesis of the potent vasodilating factor nitric oxide (NO), which is often refractory to endogenous vasoconstrictors (Titheradge, 1999). This hypotension is characterized by peripheral arterial vasodilatation, which leads to a hypodynamic status with low systemic vascular resistance, high cardiac output, and inadequate tissue perfusion, resulting in a high mortality rate (Moncada et al., 1991; Paya et al., 1993). A possible pharmacological approach to protect tissues against the deleterious effects of excessive NO production could include inhibition of NOS, for example with L-NAME or NωNLA. Etx stimulates cytokine formation and reproduces many of the cardiovascular features of sepsis in animals and humans (Pleiner et al., 2003). In animals, Etx, stimulates platelet-activating factor (PAF) and cytokines such as interleucin-1 (IL-1) and tumor necrosis factor α (TNF-α), which cause vascular relaxation and hypotension by increasing NO synthesis (Moncada et al., 1991; (Pleiner et al., 2003). NO-synthesis´inhibitors may thus be helpful in the treatment of hypotension associated with sepsis, but complete inhibition of endogenous NO synthesis may be counter-productive (Moncada et al., 1991). NO is a potent vasodilator synthesized from L-arginine by two isoforms of NO synthase in the vessel wall. One enzyme (cNOS) is always present in the vascular endothelium of animals, where it generates low NO concentrations to activate the soluble guanylate cyclase in vascular smooth muscle and regulates physiological vascular tone, blood pressure and tissue perfusion (Klosterhalfen & Bhardwaj, 1998). The second NO synthase (iNOS) is inducible in the vascular endothelium by Etx and the induction of this enzyme causes prolonged NO synthesis, which leads to sustained vasodilation and low vascular resistance to vasoconstrictors (Thiemermann, 1997). These effects can be corrected with the L-arginine analogue L-NAME, which inhibits both isoforms of NO synthase. These changes can also be prevented by pretreatment with glucocorticoids, which inhibit the expression of iNOS but do not affect the activity of cNOS (Klosterhalfen & Bhardwaj, 1998).

In this study, a single injection of a sublethal dose of Etx leads to early activation of cNOS that reaches a peak response after one hour, which is responsible for the early hyporeactivity to AII. Human umbilical vein endothelial cells incubated for 24 hours with vitamin C were protected from vasodilation and hyporeactivity (Mittermayer et al., 2005). However, the early hyporeactivity and hypotension produced after rapid i.v. administration of Etx, as in the present studies, may involve different mechanisms than those producing the persistent vascular hyporeactivity which occurs after one hour of administration of Etx. The enhanced formation of NO following the activation of cNOS present in endothelial cells mediates the immediate release of NO in response to Etx. According to Mittermayer et al. (2005) this was avoided in their experiment.

NωNLA was capable of reversing Etx-induced vascular hyporeactivity to AII in rats, as this inhibitor is selective for cNOS, the isoform involved in the rise in NO production. The reversal of the fall in MAP was due to the reduction of the synthesis of NO. The mechanism of this phenomenon remains to be clarified. On the other hand, higher doses of NωNLA inhibit both the constitutive and inducible NOS and remove all NO-dependent vasodilator tone which, in the presence of circulating vasoconstrictors during sepsis, may lead to fatal organ damage.

The reversal by AII of the effects of Etx on both the blood pressure and the vascular reactivity may be due to a sudden fall in the production of NO caused by inhibition of the enzyme or reduction of the biosynthesis of iNOS; whereas the biological half-life of NO is very short, the reversal occurs an hour after the administration of the inhibitor.

## ACKNOWLEDGEMENTS

The authors thank Rosana Finoti Pupim Silva and Elizabete Zocal Paro Lepera for technical assistance in experiments.

## RESUMO

*Hiporreatividade vascular à angiotensina II induzida pela endotoxina de Escherichia coli é revertida pelo inibidor de óxido nítrico sintase, Nω-Nitro-L-Arginina.*

**O choque séptico é uma das maiores causas de morte quando seguido de um trauma infeccioso sistêmico no homem e outros mamíferos. A sua instalação leva a uma potente hipotensão aguda e à uma redução da responsividade vascular aos agentes vasoconstritores fisiológicos e caso não sejam contidos, levam à morte. Propõe-se que estas respostas vasculares sejam devidas à**

**ação dos produtos da parede celular bacteriana liberados na corrente sangüínea pelo endotélio vascular, sendo considerado uma resposta normal de defesa do organismo contra a infecção. A redução da reatividade vascular à noradrenalina e adrenalina é observada nesta condições. No presente estudo em ratos, procurou-se avaliar se esses mesmos efeitos hipotensores e hiporreatividade também estariam relacionados com outra substância vasoconstritora endógena, a Angiotensina II (AII). Avaliou-se a variação da potência vasoconstritora da AII sobre a pressão arterial média em ratos anestesiados, antes e após a instalação da hipotensão produzida pela Endotoxina de *Escherichia coli* (Etx). Os resultados mostram que neste modelo de choque séptico, há redução da reatividade vascular à AII e esta redução pode ser revertida pelo inibidor de óxido nítrico sintase Nω-Nitro-L-Arginina (NωNLA). Os resultados sugerem que fatores endógenos associados (ainda não totalmente conhecidos), estão envolvidos na proteção dos ratos ao choque séptico, além do envolvimento da via L-arginina NO.**

*Palavras-chave:* hiporreatividade vascular; Oxido Nitrico (NO); rato; angiotensina II; NωNLA; endotoxina *Escherichia coli*.

## REFERENCES

Ambrosio AE, Fracasso JF. Effect of NωNLA or Dexamethasone on vascular hyporeactivity induced by E. *coli* endotoxin in sham and adrenalectomized rats. *Rev Ciênc Farm* 2000; 21(2):265-75.

Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RMH. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest* 1992; 101:1644-55.

Ferreira SH, Lorenzetti BB, Poole S. Bradykinin release of TNF- α plays a key role in the development of inflammatory hyperalgesia. *Br J Pharmacol* 1993; 38:c7-c9.

Flohé S, Heinrich PC, Schineider J, Wendel A, Flohé L. Tissue course of IL-6 and TNF- α release during endotoxin-induced tolerance in rats. *Biochem Pharmacol* 1991; 41:1607-14.

Fracasso JF, Nunes de Souza RL, Teixeira CE, Castro RC, Lepera EZP, Silva RFP. Effect of Dipyrone, L-NAME and L-arginine on endotoxin-induced rat paw edema. *Bras J Biol Res* 1996; 29:1543-8.

Fracasso JF, Silva RFP, Lepera EZP. Effect of NωNLA, an inhibitor of NO-synthase, on the release of tissue-plasminogem activator from rat aorta. *Rev Ciênc Farm* 2003; 24(1):23-5.

Klosterhalfen B, Bhardwaj RS. Septic shock. *Gen Pharmac* 1998; 31:25-32.

Mittermayer F. et al. Tetrahydrobiopterin corrects Escherichia coli endotoxin-induced endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 2005; 289:1752-57.

Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacolpgy. *Pharmacol* Rev 1991; 43:109-42.

Nava E, Palmer RMJ, Moncada S. Inhibition of nitric oxide synthesis in septic shock: how much is beneficial? *Lancet* 1991; 338:1555-7.

Paya D, Gran GA, Fleming I, Stocle TJ. Effect of Dexamethasone on the onset and persistence of vascular hyporeactivity induced by E. *coli* lipopolysaccharide in rats. *Circ Shock* 1993; 41:103-12.

Pleiner J, Mittermayer F, Schaller G. Inflammation-induced vasoconstrictor hyporeactivity is caused by oxidative stress. *J Am Coll Cardiol* 2003; 42(9):1656-62.

Thiemermann C. Nitric oxide and septic shock. *Gen Pharmacol* 1997; 29:159-66.

Thimermann C, Vane J. Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat *in vivo*. *Eur J Pharmacol* 1990; 182:591-5.

Titheradge MA. Nitric oxide in septic shock. *Biochim Biophys Acta* 1999; 1411:437-55.

Winner BJ, Brown DR, Michellis KM. *Statistical principles in experimental design*. 3rd.ed. New York: McGraw Hill; 1991.

Yoshikawa D. Platelet-activating factor receptor antagonist attenuates endotoxin-induced vascular hyporreactivity in the pitthed rat. *Eur J Pharmacol* 1998; 342:241-5.