Acta Veterinaria (Beograd), Vol. 56, No. 2-3, 181-189, 2006.

DOI: 10.2298/AVB0603181P UDK 619:612.124

THE EFFECTS OF ENDOTHELIN AND L-NAME ON PLASMA URIC ACID AND UREA CONCENTRATION IN WISTAR RATS

POPOVIĆ TAMARA, JOVOVIĆ ĐURĐICA, MILORADOVIĆ Z, MIHAILOVIĆ-STANOJEVIĆ NEVENA and SPASIĆ M

Institute for Medical Research, Belgrade

(Received 21. October 2005)

Effects of endothelins, which include vasoconstriction and vasodilatation, are possible by different molecular mechanisms. Endothelins induce increased synthesis of nitric oxide which in turn inhibits the synthesis of endothelin. Changes in biochemical parameters connected with the increased and decreased synthesis of nitric oxide are not thoroughly examined yet.

In a series of experiments we compared the effects of endothelin (ET-1) on the inhibition of nitric oxide synthase (NOS), as well as the effects of L-nitro arginin metal estar (L-NAME) on plasma urea and uric acid concentration. Experiments were performed on anesthetized female adult Wistar rats. The experimental groups received an i.v. bolus of ET-1, L-NAME or saline. All parameters, heamodynamic and biochemical, were measured before the animals were sacrificed.

L-NAME increased the mean arterial pressure, decreased renal blood flow and increased vascular resistance in carotid and renal arteries and increased in plasma uric acid and urea concentrations. ET-1 significantly decreased uric acid concentration in the plasma but did not effect plasma urea concentration compared to the control group. These differences show complex relations of nitric oxide with cellular signalization compared to the basic NO-cGMP pathway.

Key words: endothelin, L-NAME, haemodynamic, uric acid, urea, rats

INTRODUCTION

Endothelium as a metabolic and endocrine organ in the body is important in the modulation of vascular tonus and blood stream. It has synthetic, epicrine, autocrine and paracrine roles. Endothelial cells synthetize vasorelaxing, vasoconstricting and thromboresisting substances, as well as a number of factors and enzymes.

The most potent vasorelaxing mediator was named EDRF and was identified as endogen NO (Furchgott and Zawadzki, 1980). NO is produced by

calcium-calmodulin-NADPH dependant synthase which catalyzes the oxidation of N-guanidine terminal L-Arginine.

There are three types of this enzyme (brain NOS, endothelial NOS, inducible NOS) (Moncada and Higgs, 1995; Snyder, 1995). L-NAME (L-nitro-arginine methyl estar) is a blocker of NOS and induces vasoconstriction. NO induces smooth muscle cells to relax, via cGMP kinase which decreases intracellular Ca^{2+} level and relaxes smooth muscle cells by defosforilation of myozin light chains.

The endothelins (ETs) belong to a family of potent vasoconstricting peptides that were first isolated from vascular endothelial cells in 1988 (Yanagisawa *et al*. 1988). The three isoforms of ETs (ET1, ET2, ET3) are produced in the renal tissue (Kohan, 1993).

ET-1 has been described as a very powerful vasoconstricting agent isolated from vascular endothelium (Battistini *et al*., 1993). ETs exert their effects via two types of receptor, classified as ETa and ETb (Chandrashekar *et al*., 1994). It is well known that endothelins stimulate endothelial NO synthase which results in increasing NO concentration as a secondary messenger. Urea is synthesized in the urea cycle and during NO synthase blockade, urea production decreases. Uric acid is formed from degradation of purine bases by xanthine oxidase.

The aim of our study was to compare effects of ET-1 and L-NAME on heamodynamic and biochemical parameters, specially uric acid and urea concentration changes in Wistar normotensive rats.

MATERIALS AND METHODS

Female Wistar rats, weighting about 200 g, bred at the Institute for Medical Research, Belgrade were fed on standard chow for laboratory rats (Veterinarski zavod, Subotica, Serbia and Montenegro). All animal experiments were conducted in accordance with local institutional guidelines for the care and use of laboratory animals. The investigation also conformed to the principles and guidelines of the Canadian council on Animal Care (CCAC). For the treatment of experimental animals in ous study we used the vasoconstrictor 41199-Endothelin (rat) 380804, L-NAME as well as saline.

Experimental procedure and groups

All our experiments were performed in anaesthetized (35 mg/kg b.m. sodium pentobarbital, i.p.) rats. The animals were divided into three groups: control group (n=10) saline infused, L-NAME (n=10) group (i.v. bolus – 10 mg/kg) and ET-1 (n=10) group (i.v. bolus – 0.5 mg/kg). Right femoral artery and left femoral vein were cannulated by poliethylen catheter (PE-50, Clay-Adams, Parsippany, NJ, USA). Systolic, diastolic blood pressure and heart rate (direct registrator - Physiograph four, Narco Bio System, Inc) were measured, while mean blood pressure was calculated by electronic integration. All parameters were measured before the rats were sacrificed.

Heamodynamic measurements

The dissolved substances were applied intravenously in a bolus by a catheter implanted in the left femoral vein. Then mean arterial blood pressure (MAP) and heart rate were measured. Blood pressure values were measured after giving anesthesia (MAP1, 2) as well as after the application of tested substances (MAP 3,4).

For blood flow measurements the carotid and renal arteries were gently separated. An ultrasonic flow probe (1RB, internal diameter=1 mm) was placed around the arteries for the measurement of renal blood flow (RBF) and carotid blood flow (CBF), using a Transonic T106 Small Animal Flowmeter (Transonic System Inc., Ithaca, NY, USA). Renal vascular resistance (RVR) and carotid vascular resistance (CVR) were calculated by dividing MAP by renal blood flow and carotid blood flow (Hg/kg/μL. b.m.).

Biochemical measurements

Before sacrificing the animals we took blood from the abdominal aorta. Plasma concentration of urea and uric acid were determined by commercial kits using a spectrophotometer Cobas Mira, Rosh (Elitech Diagnostic).

Statistical analyses

The results are expressed as mean ±SEM. One-way analysis of variance (ANOVA) was applied. When the ANOVA results were significant, Bonferroni's t-test was used to determine level of significance and p value <0,05 was considered to be statistically significant (Primer of Biostatistics, by Stanton A. Glanz).

RESULTS

Heamodynamic parameters

MAP was significantly increased ($p<0.05$) in the group infused with L-NAME compared to the control; ET-1 markedly increased MAP but not significantly (Fig.1).

Carotid heamodynamic parameters

In the carotid vascular bed MAP and CVR were significantly increased while CBF was markedly decreased in the L-NAME group compared to the control. ET-1 infused group showed an increase of MAP and CVR but no changes in CBF values compared to the control group (Fig. 2).

Renal heamodynamic parameters

MAP and RVR were significantly increased and RBF was decreased in L-NAME group compared to the control. ET-1 group showed no statistical differences of the same parameters, compared to the control (Fig. 3).

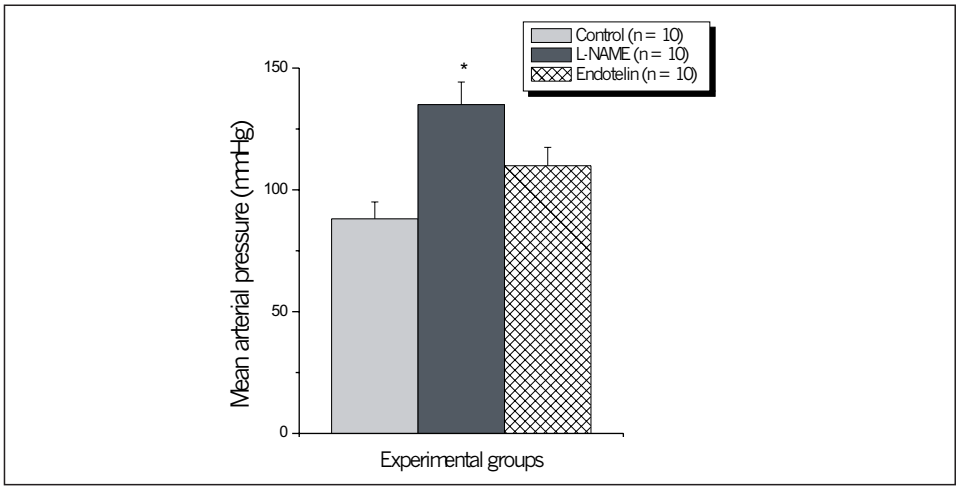

Figure 1. Mean arterial pressure after substance infusion in experimental groups.
*p <0.05 compared to control group; n – number of animals

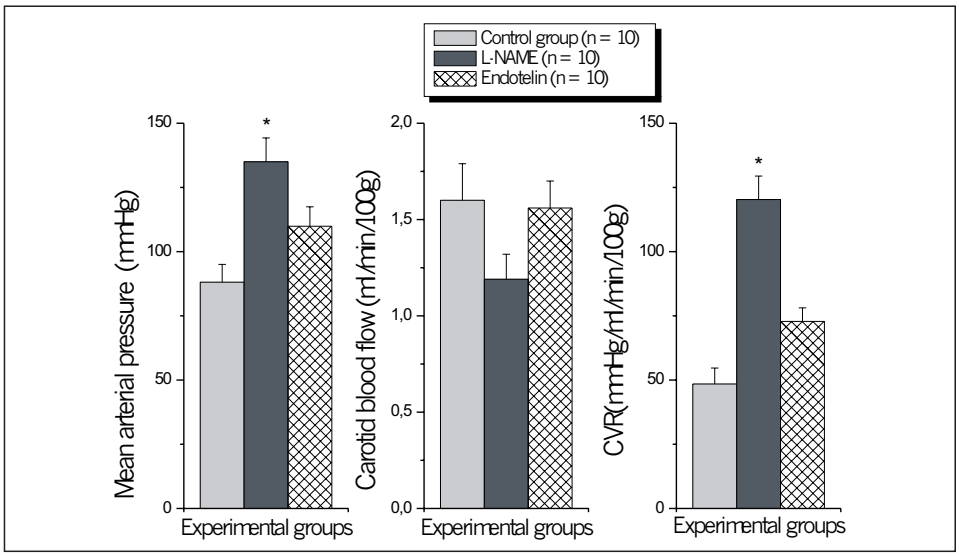

Figure 2. Mean arterial pressure, carotid blood flow and carotid vascular resistance (CVR) in experimental groups.
*p<0.05 compared to control group; n – number of animals

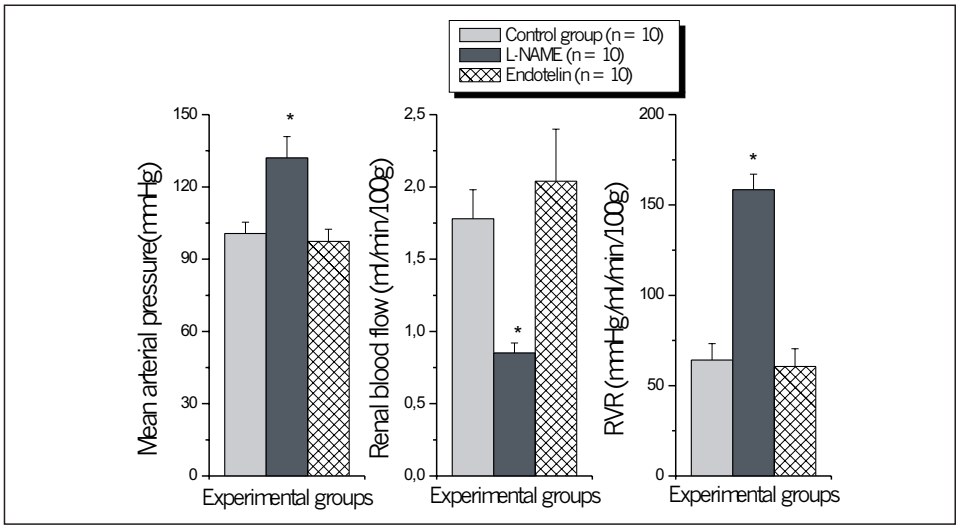

Figure 3. Mean arterial pressure, renal blood flow and renal vascular resistance (RVR) in experimental groups.
**p*<0.05 compared to control group; *n* – number of animals

Biochemical parameters

In the L-NAME and ET-1 group plasma urea concentration was significantly increased compared to the control group (Fig. 4).

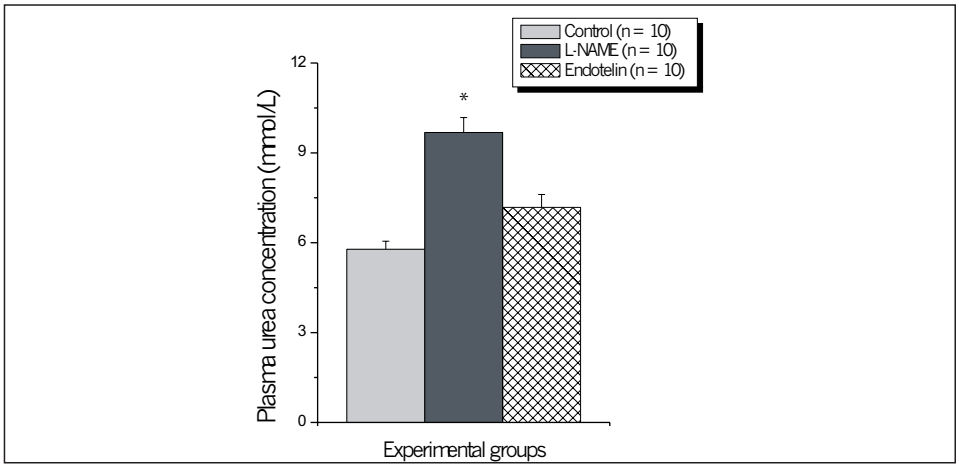

Figure 4. Plasma urea concentration in experimental groups.
**p*<0.05 compared to control group; *n* – number of animals

Plasma uric concentration was significantly increased in L-NAME group compared to the control group. ET-1 group showed a significant decrease in the concentration of plasma uric acid when compared to the control group (Fig 5).

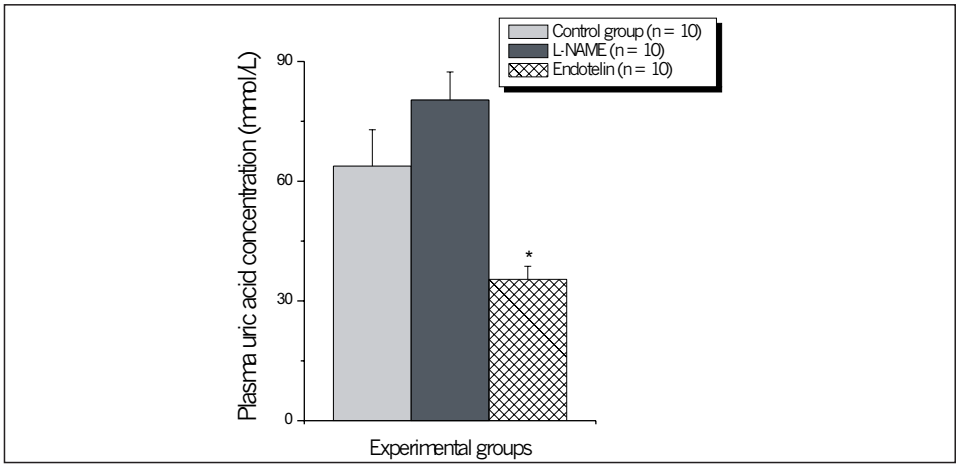

Figure 5. Plasma uric acid concentration in experimental groups.
*$p < 0.05$ compared to control group; n – number of animals

DISCUSSION

Our study showed that a short-time inhibition of nitric oxide biosynthesis with L-NAME, induces an increase in mean arterial pressure. Regional heamodynamic responses to inhibition of nitric oxide synthesis showed decreasing vascular blood flow and increasing vascular resistance in both examined vascular beds in treated animals.

Acute inhibition of conversion L-arginine into nitric oxide leads to increasing arterial pressure and TVR as well as decreasing cardiac output (Loeb & Longnecher, 1992; Rees *et al.*, Wang *et al*., 1992). Increasing in TVR due to increasing resistance of many vascular beds including renal (Baylis *et al*., 1990; Beierwaltes *et al*., 1992; Gardiner *et al*., 1990b; Loeb& Longnecher, 1992; Wang *et al.*, 1992), mesenteric (Gardiner *et al*., 1990b), cerebral (Loeb & Wang *et al*., 1992), gastric (Pique *et al*., 1989) and splenic (Loeb & Longnecher, 1992; Wang *et al.*, 1992) circulation showed that the increase of resistance is not equal in all vascular beds.

All of this suggests that nitric oxide has an important role in the distribution of blood flow. That degree of its influence to a specific flow-resistant domain can be an indicator of how much the local perfusion in such domain depends on nitric oxide presence.

In spite of the classical hypothesis in which the cause of hypertension is perceived in connection to misbalance of vasoconstrictor influences, the new

concept in the regulation of arterial pressure understands the existence of balanced synthesis of vasoconstricting and vasodilatating factors (Luscher, 1990). It is well known that endothelin stimulates endothelial NOS which results in the increase of nitric oxide in the circulation which in turn starts a cascade of events (NO as a secondary messenger).

Results of our study showed that intravenous infusion of endothelin induced an initial decrease of arterial blood pressure which soon started to increase and come to its maximum values ten minutes after infusion. Randy *et al*. (1996) showed that ET-1 infusion (0,5 nmol/kg i.v.) in NW rats induced an initial decrease of arterial blood pressure.

Further examinations of endothelial receptors (ETa & ETb) showed that ETa receptor blockade induced significant vasodilatation which is reduced by about 95% with simultaneous inhibition of endogenous nitric oxide production. ETb blockade alone, or with ETa receptor antagonist, induced vasoconstriction and showed that ETb receptors dominantly interfere ET-1 induced vasodilatation (Rossi *et al*., 2001).

Experimental models also assured that ET-1 contributed to the physiological regulation of vascular tonus by stimulation of nitric oxide production via ETb receptors. The conclusion was that vasoconstricting effects can be more effective in reduced nitric oxide activation conditions, such as hypertension and arteriosclerosis (Rossi *et al*., 2001). ET-1 interacted with non endothelial pathways: simpatical system, renin-angiotensin system and CNS. Although *in vitro* studies showed interaction of ET-1 in these systems, there is no direct evidence that ET-1 is involved in hypertension pathogenesis or *in vivo* vascular tonus regulation. Several groups which examined hypertension perceived an increase in ET-1 level during moderate hypertension, while other studies did not cconfirm such findings (Sitki *et al*., 1996). There is a conclusion that degradation of endothelin is tissue specific. Although its metabolism is rapid, endothelin is either bound to ETa receptor on cells or it interacts with membrane lipids which have a protective role from protease activation. The later could explain the role of endothelin as a potent factor in muscle cell contractions (Bermek *et al*., 1996).

The obtained results clearly demonstrate the difference in plasma uric acid but not urea concentration between these experimental groups. Endothelin (ET-1) infusion probably via ETb receptors induces a short time increase in renal blood flow and consequently the decrease of uric acid concentration due to increased glomerular filtration rate. The vascular NO-dependant physiological response may be the consequence of three different molecular events:

– NO stimulation of soluble guanylate cyclase and modulation of mitochondrial respiration,

– NO induced S-nitrosylation, including inhibition of caspases and

– Intracellular formation of peroxynitrite and the activation of nitrogen-activated protein kinases (Levonen *et al*., 2001).

In our experiment we compared the effects of ET-1 induced ETb endothelial NO production with L-NAME inhibition in NO production and detected opposite effects on uric acid concentration but not on urea concentration. Although urea

production should be closely related to NOS activity these differences indicate a more complex relation of NO with cell signaling than the basic NO-cGMP relation.

Address for correspondence:
M.Sc Tamara Popović
Institute of medical research
Dr Subotića 4, PO Box 102
11129 Belgrade, Serbia & Montenegro
e-mail: poptam@imi.bg.ac.yu

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UTICAJ ENDOTELINA I L-NAME NA KONCENTRACIJU MOKRAĆNE KISELINE I UREE U PLAZMI KOD WISTAR PACOVA

POPOVIĆ TAMARA, JOVOVIĆ ĐURĐICA, MILORADOVIĆ Z,
MIHAILOVIĆ-STANOJEVIĆ NEVENA i SPASIĆ M

SADRŽAJ

Efekti endotelina koji uključuju vazokonstrikciju I vazodilataciju ostvaruju se različitim molekularnim mehanizmima. Endotelin indukuje povećanu sintezu azotnog oksida za koji se smatra da povratno inhibira sintezu endotelina. Promene biohemijskih parametara vezanih za povećanu i smanjenu sintezu NO nisu dovoljno proučene. U ovom radu su upoređeni efekti endotelina (ET-1) i inhibitora NOS-a, L-NAME na koncentraciju mokraćne kiseline i uree u plazmi. Eksperimenti su izvedeni na anesteziranim odraslim NW pacovima, ženskog pola. Eksperimentalne životinje su u i.v. bolusu primale ET-1, L-NAME ili fiziološki rastvor. Svi hemodinamski i biohemijski parametri su mereni pre žrtvovanja. L-NAME dovodi do povećanja srednjeg arterijskog pritiska, smanjenja renalnog protoka i povećanja vaskularnog otpora u karotidnoj i renalnoj arteriji i povećanja koncentracije mokraćne kiseline i uree u plazmi. ET-1 značajno smanjuje koncentraciju mokraćne kiseline u plazmi ali ne utiče na koncentraciju uree u odnosu na kontrolu.

Ove razlike pokazuju kompleksnije relacije NO-a sa ćelijskom signalizacijom u odnosu na osnovni NO-cGMP put.