Pharmacological Reports
2012, 64, 511–520
ISSN 1734-1140

Review

L-NAME in the cardiovascular system – nitric oxide synthase activator?

Jana Kopincová[1,2], Angelika Púzserová[2], Iveta Bernátová[2]

[1]Department of Physiology, Jessenius Faculty of Medicine, Comenius University, Malá Hora 4A, 036 01 Martin, Slovak Republic

[2]Institute of Normal and Pathological Physiology, Centre of Excellence for Examination of Regulatory Role of Nitric Oxide in Civilization Diseases, Slovak Academy of Sciences, Sienkiewiczova 1, 813 71 Bratislava, Slovak Republic

Correspondence: Jana Kopincová, e-mail: jana.kopincova@jfmed.uniba.sk

Abstract:
L-arginine analogues are widely used inhibitors of nitric oxide synthase (NOS) activity both *in vitro* and *in vivo*, with N^{ω}-nitro-L-arginine methyl ester (L-NAME) being at the head. On the one hand, acute and chronic L-NAME treatment leads to changes in blood pressure and vascular reactivity due to decreased nitric oxide (NO) bioavailability. However, lower doses of L-NAME may also activate NO production *via* feedback regulatory mechanisms if administered for longer time. Such L-NAME-induced activation has been observed in both NOS expression and activity and revealed considerable differences in regulatory mechanisms of NO production between particular tissues depending on the amount of L-NAME. Moreover, feedback activation of NO production by L-NAME seems to be regulated diversely under conditions of hypertension. This review summarizes the mechanisms of NOS regulation in order to better understand the apparent discrepancies found in the current literature.

Key words:
L-arginine analogues, N^{ω}-nitro-L-arginine methyl ester, nitric oxide synthase, feedback regulation, nuclear factor κB

Abbreviations: BHR – borderline hypertensive rats, EDRF – endothelium-derived relaxing factor, eNOS – endothelial nitric oxide synthase, iNOS – inducible nitric oxide synthase, L-NA – N^{ω}-nitro-L-arginine, L-NAME – N^{ω}-nitro-L-arginine methyl ester, L-NMMA – N^{ω}-monomethyl-L-arginine, NF-κB – nuclear factor κB, NO – nitric oxide, NOS – nitric oxide synthase, ROS – reactive oxygen species, SHR – spontaneously hypertensive rats, WKY – Wistar-Kyoto rats

Introduction

The great and well-known nitric oxide (NO) story began in 1987, when two experimental groups [31, 50] finally identified chemical basis of endothelium-derived relaxing factor (EDRF) discovered formerly by Furchgott and Zawadzki in 1980 [21]. After 6-year long hard work of identifying EDRF, one year sufficed to reveal the intracellular source of NO, L-arginine [52] and at the same time, L-arginine analogue, N^{ω}-monomethyl-L-arginine (L-NMMA), which had been shown to inhibit NO-synthesizing enzyme [51].

Discovery of three types of NO synthase (NOS) in early 90's has brought interest to developing various inhibitors of NO synthesis. Soon, L-arginine analogues became widely used NOS inhibitors, providing useful tool for achieving NO-deficient conditions.

L-arginine analogues

Analogues of L-arginine serve as NOS inhibitors by virtue of their substitution at one or both of the terminal guanidino (^{G} or $^{\omega}$) nitrogen [68]. Generally, L-isomers solely are considered to be active inhibitors, although there is also evidence that N^{ω}-nitro-D-arginine might inhibit endothelial relaxation of the aortal rings and N^{ω}-nitro-D-arginine methyl ester inhibited NOS activity both in the rat heart and aorta [5, 83].

The mechanism of NOS inhibition by substrate analogues consists in competitive bonding to enzyme, but the molecular background of inhibitory action varies for particular analogues. In most cases, analogue-mediated inhibition of NO synthesis is *in vivo* reversible after L-arginine replenishment. However, there are some substances, such as L-NMMA, N^{ω}-iminoethyl-L-ornithine or N^{ω}-allyl-L-arginine that can be utilized by NOS forming intermediates tightly bound to enzyme molecule and thereby blocking the synthesis irreversibly – that is why they deserved the name "suicide inhibitors" [49, 68].

Reversible inhibitor and one of the most frequently used L-arginine substituents is N^{ω}-nitro-L-arginine (L-NA), or its esterified form N^{ω}-nitro-L-arginine methyl ester (L-NAME), which are considered to be non-selective inhibitors [43, 74]. Esterification of the carboxyl group of L-NA increases water solubility, which simplifies the experimental use of this analogue. On the other hand, specific esterase is needed to fully exhibit inhibitory action in the tissue and that may constrain the effect of L-NAME for particular tissues [27]. Despite 30–100-times lower inhibitory efficiency compared to L-NA, L-NAME is widely used in both acute and long-term *in vitro* and *in vivo* experiments, when the effects of NO production restriction are investigated.

L-NAME administration

In endothelium, NO production is continual, keeping balance between vasoconstriction and vasodilatation. NO acts as an antagonist of various constrictoric factors with sympathetic nervous system at the head [2]. Therefore, changes in arterial blood pressure or total peripheral resistance after acute L-NAME administration into vascular bed can be very quickly noticed and the intensity of reaction is determined by both the amount of L-NAME in the dose and the rate of organism dependence on NO [15, 29]. Similarly, application of both L-NAME and L-NMMA led to dose-dependent constriction of the isolated rings of the rat aorta and femoral artery *in vitro* [64].

The theory of "endothelial dysfunction", and consequential insufficient NO production in human essential hypertension, led to creation of an animal model of human hypertension due to NO deficiency, achieved by long-term L-NAME treatment of experimental animals. Long-term administration of NOS inhibitor in relatively high doses (10, 20, 25, 40, 50, 65, 80, 100 mg per kg of body mass per day) induced so-called "NO-deficient hypertension" in normotensive rats and this model became widely used tool for investigation of the NO participation in cardiovascular disorders [3, 11, 13, 18, 19, 35, 40, 48, 66, 86]. Nevertheless, the way of drug administration, the dose as well as the treatment period varies from case to case and sometimes ends in arguable findings, as the effects of L-NAME treatment on blood pressure may be inconsistent even in one laboratory using the same rat strain (compare [40, 48] and [77]).

Another controversial matter of long-term L-NAME treatment is L-NAME-induced left ventricular hypertrophy. In some cases left ventricular hypertrophy was present after 3-week-long treatment with the L-NAME in the dose of 10 mg/kg/day, but, on the other hand, hypertrophy was absent even after 8 weeks of 100 mg/kg/day L-NAME administration (Tab. 1).

Regarding vascular function, higher doses of L-NAME administered for 3–6 weeks reduced relaxant response of the aorta, femoral artery and small mesenteric arteries to carbachol or acetylcholine *in vitro* [10, 19, 39, 57]. In addition, the doses of 10 to 100 mg of L-NAME per kg/day led to dramatic decrease of cGMP content in the aorta of normotensive rats [4, 13, 26].

One of very specific cases is an administration of L-NAME to rat strain with spontaneous or borderline hypertension. These spontaneously hypertensive rats (SHR) develop blood pressure up to 180–200 mmHg in the 4^{th} – 6^{th} week of life on genetic basis without any pharmacological or physiological intervention, regardless of sodium diet [69]. Borderline hypertensive rats (BHR) are F1 offspring of one normotensive and one spontaneously hypertensive parent. Such genetic combination becomes manifested by blood pressure elevation to levels about 130–150 mmHg [8, 67]. Previously, SHR were thought to have impaired

Tab. 1. The effect of different dose of L-NAME and the treatment duration on the development of left ventricular hypertrophy in normotensive rats

Dose (mg/kg/day)	Treatment duration	Strain	Hypertrophy	Reference
10	3 weeks	Wistar	A	77
10	3 weeks	WKY	P	19
10	4 weeks	Wistar	A	4
20	3 weeks	Wistar	A	77
20	25 days	Wistar	P	35
20	4 weeks	Wistar	P	58
20	4 weeks	Wistar	A	4
20	8 weeks	Wistar	P	20
40	4 weeks	Wistar	P	5, 10
40	5 weeks	Wistar	P	57
40	6 weeks	Wistar	P	72
50	4 weeks	Wistar	A	4
50	5 weeks	Wistar	P	56
50	8 weeks	Wistar	A (19 from 25)	3
50	8 weeks	Wistar	P (6 from 25)	3
~60	8 weeks	Wistar	P	87
100	4 weeks	Wistar	A	4
100	8 weeks	Wistar	A	44

Legend: A – absent, P – present, WKY – Wistar-Kyoto rats

NO production because of endothelial dysfunction observed in this strain [62, 85]. Later, many experiments showed elevated basal NO production in SHR [25, 63]. However, the organism dependence on NO in SHR is much higher than in normotensive strains supposedly due to elevated oxidative stress resulting in elevated NO turnover. Thus, in SHR L-NAME administration of 25 mg/kg/day for just 15 days had fatal consequences [1]. Moreover, 4-week L-NAME administration of 8–12 mg/kg/day led in SHR to blood pressure elevation up to 30–40 mmHg, increase in total peripheral resistance, myocardial hypertrophy and diminished relaxation of the mesenteric vascular bed or the aorta to carbachol or isoprenaline, together with significant morbidity and mortality [6, 7, 47, 73, 78]. Within 4 weeks of ~8 mg/kg/day of L-NAME, only 42% of SHR survived and prolongation of treatment up to 2 months resulted in 100% morbidity/mortality in SHR [47]. Furthermore, in our experiments, administration of L-NAME in the low dose of 1.5 mg/kg/day to SHR caused the death of animals within 2–4 weeks and marked blood pressure elevation was observed even after the dose of 0.1 mg/kg/day in 2 weeks, while similar doses had no effect on blood pressure in normotensive rats [36].

Similar discrepancy was found in normotensive rats and BHR after 4 weeks of approximately 17 mg/kg/day L-NAME administration (drinking of L-NAME solution with concentration 150 mg/l). The dose, which was tolerated by normotensive rats without any consequences after the withdrawal of treatment, led to significant blood pressure elevation in BHR even after the termination of drug administration, in some cases associated with lethargy, suppressed appetite and loss of motor function in the pelvic appendages followed by the death [71]. Blood pressure reaction of BHR to L-NAME administration can be seen even after the dose of 1 mg/kg/day within 3 weeks, while at similar dose normotensive rats remain unaffected [12, 36].

This short summary indicates that the response of normotensive and hypertensive organism to the same dose of L-NAME differs and, apparently, the sensitivity of NOS/NO pathway is altered under hypertensive conditions [63, 70].

The feedback phenomenon of long-term L-NAME treatment

While L-NAME administration aimed at lowering of NO production is widely used, much less is known about the feedback consequences of long-term L-NAME treatment on NO production.

The phenomenon of feedback regulation of nitric oxide synthase expression and activity by the level of produced NO *in vitro* has already been observed in 1992 (for more details see [37]). Briefly, under physiological conditions, NO produced by NOS binds to heme iron of NOS molecule and thus inhibits synthetic activity of the enzyme. The rate of inhibition depends on isozyme of NOS. Accordingly, lowering NO levels in reaction solution by NO scavenging (e.g. by oxyhemoglobin) led to maintaining of synthetic activity, while adding NO donor to the solution gradually slowed down NO production [65]. In the same direction, expression of particular isoform of NOS is regulated by NO production. Preincubation of cell culture with exogenous NO donor led to attenuated expression of both inducible (iNOS) and endothelial NOS (eNOS) isoforms [16, 81]) and, on the contrary, the presence of NO scavenger increased iNOS expression after cytokine stimulation [53].

The presence of L-NAME acts on NOS activity and expression in a similar manner like NO scavenging. The presence of L-NAME in cytokine stimulated cell line led to both heightened expression and activity of iNOS [53] and, interestingly, incubation of the endothelial cells with L-NAME causes 140-percent enhancement in eNOS protein level as an *in vitro* response to shear stress, which is a natural stimulus for NO synthesis *in vivo* [23]. However, it is rarely considered that the same effect on NOS expression or activity may be reached in long term L-NAME treatment.

L-NAME and NOS activity

The first *in vivo* observation of L-arginine analogue-induced improvement of NO production was made by 7-day-lasting administration of 300 mg/kg/day of L-NMMA to Sprague-Dawley rats *via* continuous infusion through the portal vein by an osmotic pump [42]. As a result, increased iNOS expression in the liver tissue and 38-percent elevation in iNOS activity were achieved.

It is quite surprising that an agent used for NOS inhibition may have opposite effects on NOS activity. However, there is a lot of discrepancies in experimental results. Not only in our laboratory, decreased NOS activity was repeatedly found after 4-week L-NAME administration of 40 mg/kg/day in the aorta, several brain parts, left ventricle or kidney [10, 11, 13, 58, 77], which was associated with worsen relaxant response of the aorta, renal and pulmonary artery to acetylcholine [28] – that is, after all, the original aim of the L-NAME usage.

By contrast, 7 weeks of L-NAME administration in the dose of 40 mg/kg/day to normotensive Wistar-Kyoto (WKY) rats led to increased NOS activity in the rat heart, aorta and kidney [59]. Amazingly, at the same dose of L-NAME, the treatment period and the rat strain, these authors found no change in NOS activity in another experiment [82], while decrease in NOS activity in the left ventricle and kidney of Wistar rats after 5 and 6 weeks of 40 mg/kg/day L-NAME treatment was found, together with impaired acetylcholine-induced relaxation of the femoral and small mesenteric arteries [55, 72]. Thus, it remains unanswerable, why feedback regulation did not exhibit in one of experiments, and whether one week may serve as "breaking point" in the molecular response to inhibitor or it is just a difference between two rat strains.

Aimed at verifying the existence of L-NAME-induced feedback regulation of NOS activity *in vivo*, we made several experiments on long-term L-NAME treatment, using very low doses of L-NAME up to 0.3 mg/kg/day and prolonged treatment period (4 to 10 weeks). In the first experiment, normotensive Wistar rats were treated by L-NAME in the dose of 1.5 mg/kg/day for 8 weeks and such treatment led to increased NOS activity in the left ventricle and aorta associated with improved response of the femoral artery to acetylcholine, while the blood pressure reaction to L-NAME was only transient [9]. However, at the same dose we did not find any elevation in NOS activity in BHR with Wistar father [38]. Recommended by reviewers, we switched to another normotensive strain – WKY rats – and tried to lower the dose with the aim to prevent the transient blood pressure elevation. Ten weeks of L-NAME administration of 0.3 and 1 mg/kg/day did not affect blood pressure, but increased NOS activity in both the left ventricle and aorta. Lowering of the dose and the strain change showed up in NOS activity response to L-NAME treatment also in BHR with WKY father. After 10-

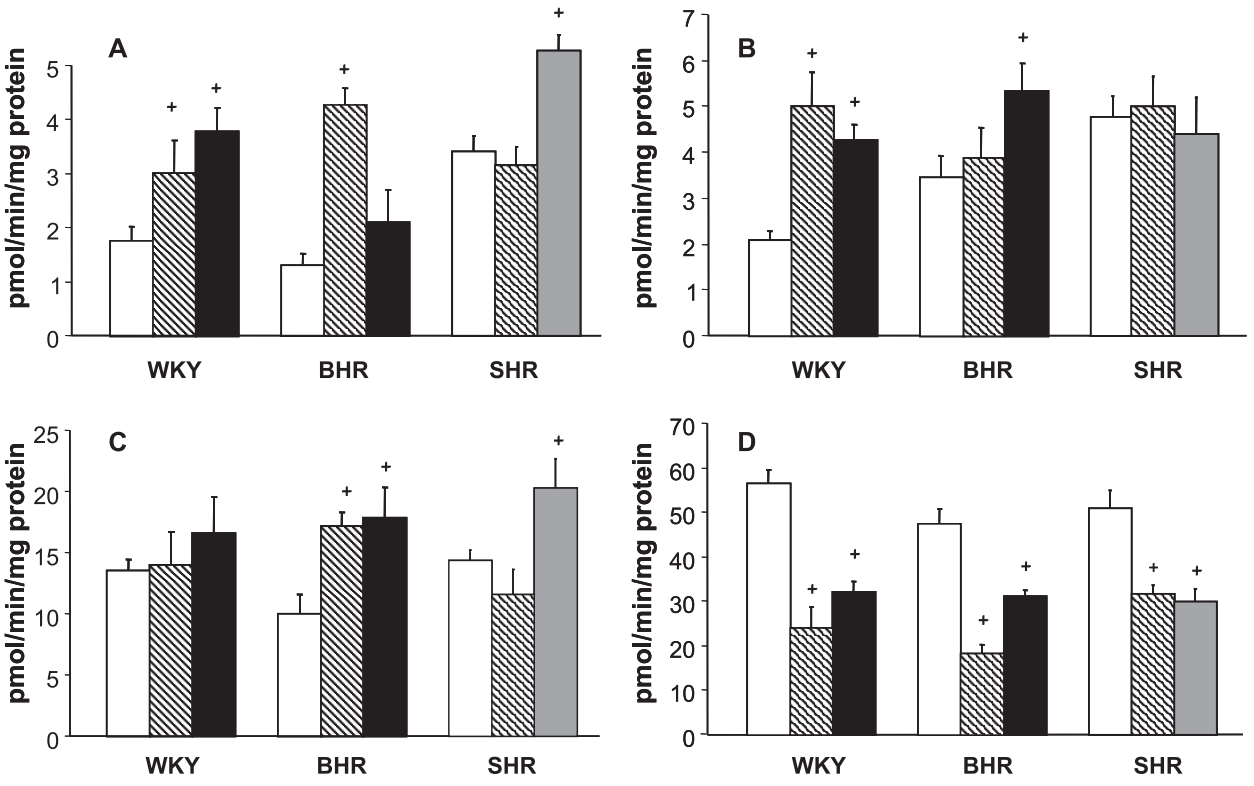

Fig. 1. The effect of ten week lasting low dose L-NAME treatment on NOS activity in the left ventricle (**A**), aorta (**B**), kidney (**C**) and hypothalamus (**D**) of rats. White columns stand for controls, crosshatched columns stand for L-NAME dose 0.3 mg/kg/day, grey columns stand for L-NAME dose 0.1 mg/kg/day and black columns stand for L-NAME dose 1 mg/kg/day for 10 weeks. Total NOS activity was measured in tissue homogenates by determination of [^{3}H]-L-citrulline formation from [^{3}H]-L-arginine, as described previously [63] and expressed as pmol/min/mg of tissue proteins. $^+$ $p < 0.05$ compared to untreated controls; WKY – Wistar-Kyoto rats, BHR – borderline hypertensive rats, SHR – spontaneously hypertensive rats [36]

week treatment, the left ventricular and renal NOS activity were elevated within the dose of 0.3 mg/kg/day and aortal and renal NOS within 1 mg/kg/day of L-NAME (Fig. 1A, B, C [36]). However, comparing the effect of 4 and 10 weeks of 1 mg/kg/day of L-NAME treatment on NOS activity, we found that after 4 weeks there was reduction in NOS activity in the hypothalamus, aorta and liver [12] (see Tab. 2). Thus it seems that feedback-regulated activation of NOS activity *in vivo* requires specific period of time.

When trying to evoke such feedback-induced NOS activity improvement in SHR, we were forced to additional dose cut-down, as SHR were not able to cope with 1 mg/kg/day of L-NAME. Thinking about the doses up to 100 mg/kg/day, it seemed irrational to expect any results when treating SHR with the dose of 0.1 mg/kg/day. However, such low amount of L-NAME managed to elevate NOS activity in the left ventricle and kidney of SHR (Fig. 1A, C [36]). So the results suggest that feedback control of NOS activity works both in normotensive and hypertensive rats. However, the sensitivity of such regulation showed between-strain and between-organ differences.

The only agreement in long-term L-NAME treatment effect consists in observation of decreased NOS activity in selected parts of the central nervous system. Administration of the dose from 0.1 or 1 mg/kg/day to 40 mg/kg/day for 4 to 10 weeks caused rather inhibition than feedback-associated enhancement in brain NOS activity in both normotensive and hypertensive strains. This event was seen in spite of feedback NOS activation exhibited in the peripheral tissues, suggesting that there is strong difference between NOS activity regulation in the central nervous system and in the peripheral organs or tissues [32, 36, 59, 77] (Fig. 1D).

Tab. 2. The effect of 4 and 10 weeks of L-NAME administration in the dose of 1 mg/kg/day on NOS activity in various tissues of BHR compared to age-matched BHR control

NOS activity	L-NAME 4 weeks	L-NAME 10 weeks
Hypothalamus	↓	↓
Aorta	↓	↑
Liver	↓	↑
Kidney	0	↑

Legend: Direction of change is indicated: ↓ – decrease in NOS activity, ↑ – increase in NOS activity, 0 – no change in NOS activity [12]

L-NAME and NOS expression

Considering that L-NAME administration blocks NO synthesis, we can suppose that higher NOS activity found in particular tissues *ex vivo* results from higher level of NOS gene expression. Indeed, the presence of L-NAME increases *in vitro* the rate of both iNOS and eNOS expression [23, 53], perhaps *via* influencing the balance between NO and transcription factor nuclear factor κB (NF-κB) which is key factor in NOS expression regulation [23, 30, 37]. NO is considered to inhibit either NF-κB activation [16], its binding to DNA [54] or to induce and stabilize NF-κB inhibitor [17, 61], and thus to modulate the rate of NOS transcription. In this sense, the decrease in NO level induced by L-NAME leads to NF-κB activation and consequently to increased NOS expression.

Paradoxically, it seems that the effect of L-NAME administration *in vivo* on NOS expression may differ depending on the treatment period and the tissue investigated. Forty mg/kg/day of L-NAME for 4 weeks significantly increased eNOS and iNOS expression in Sprague-Dawley rat cavernous tissue [24] and eNOS in the Wistar rat heart and kidney, but it did not affect eNOS expression in the rat brain [59, 60]. However, the same dose of L-NAME did not affect eNOS or iNOS expression in Wistar aorta after 5 weeks of treatment [55], while 7 weeks of treatment potentiate eNOS expression increase in the rat heart and, contrary, decreased eNOS expression in the rat brain [59].

Nevertheless, L-NAME-induced feedback regulation of NOS expression is more complicated than regulation of NOS activity. We have to reason out that activation of NF-κB may be evoked by several factors, more of which are somehow associated with NO production.

Firstly, oxidative load induces NF-κB activation in the same way as NO deficiency and the presence of reactive oxygen species (ROS) may thus evoke higher eNOS or iNOS expression [22, 88]. Thereby, it is sometimes impossible to resolve, whether the increase in NOS expression is caused by NO depletion or rather by ROS-mediated NF-κB activation. Moreover, under conditions of oxidative stress, some of NOS cofactors turn inactive and NOS enzyme synthesizes rather superoxide radical than NO [14] which yet more worsen the vasoactive balance.

In addition, high-dose of L-NAME treatment (at least 40 mg/kg/day) is often associated with the elevated level of secondary products of oxidative stress [55, 82], and the same goes for spontaneous and borderline hypertension [33, 41, 75, 84]. This may be the background of higher eNOS expression, which was found repeatedly in BHR and in SHR even in the prehypertensive age of 3 weeks, suggesting that elevated blood pressure *per se* is not the cause of eNOS expression increase [79, 80]. Hence, marked oxidative load after administration of high dose of L-arginine analogues may also be responsible for activation of iNOS expression and through that also iNOS activity in above mentioned experiment with 300 mg/kg/day of L-NMMA to Sprague-Dawley rats [42].

Secondly, NF-κB activation in the vascular tissue is either induced by shear stress [23], which is better known to activate eNOS *via* its phosphorylation [34]. But, however, shear stress may be accentuated due to blood pressure elevation after L-NAME administration and thus it may activate NF-κB, which in turn may lead to increased eNOS transcription as well.

Nevertheless, increased NOS expression need not be associated with better NO bioavailability *in vivo*. Under conditions of higher oxidative load, cofactors needed for NO synthesis, especially tetrahydrobiopterin, may be oxidized and thus inactive [45] and NO may be scavenged by ROS [46, 75]. Despite marked NOS expression, *in vivo* activity of NOS could hence be diminished resulting in reduced NO bioavailability. Thus, it is worthy to mention that this disparity between NOS expression and activity entails pitfall in determination of NOS activity *in vitro*, as this method always requires adding of external cofactors to the reaction mixture and thus may reveal enzymatic activity, which is absent *in vivo*. On that score, blood pressure in high-dose L-NAME-treated rats often re-

mains elevated despite observed NOS activation *in vitro* [59, 76] and likewise, vascular responses to vasodilatory agents often fail to exhibit any improvement [76].

In conclusion, this short review of literature on the effects of L-arginine analogues, especially L-NAME, showed that regulation of NO production *in vivo* is precisely regulated, but not precisely known process. Disruption of this regulation by using apparent inhibitors of NO production and modulation of NO levels might paradoxically increase NOS expression or NOS activity depending on conditions of experiment – mainly on duration of treatment and dose of L-NAME, rat strain, tissue investigated as well as isoform of NOS. This should be always taken into consideration when analyzing and discussing results regarding the role of NO in physiological and pathophysiological conditions.

Acknowledgments:
This study was supported by Project "Center of Excellence for Perinatology Research" No. 26220120016 co-financed from EU sources, by Grant APVV-0523-10 and Grant VEGA No. 1/0291/12.

References:

1. Arnal JF, Battle T, Menard J, Michel JB: The vasodilatory effect of endogenous nitric oxide is a major counter-regulatory mechanism in the spontaneously hypertensive rat. J Hypertens, 1993, 11, 945–950.
2. Arnal JF, Dinh-Xuan AT, Pueyo M, Darbladea B, Rami J: Endothelium-derived nitric oxide and vascular physiology and pathology. Cell Mol Life Sci, 1999, 55, 1078–1087.
3. Arnal JF, el Amrani AI, Chatellier G, Ménard J, Michel JB: Cardiac weight in hypertension induced by nitric oxide synthase blockade. Hypertension, 1993, 22, 380–387.
4. Arnal JF, Warin L, Michel JB: Determinants of aortic cyclic guanosine monophosphate in hypertension induced by chronic inhibition of nitric oxide synthase. J Clin Invest, 1992, 90, 647–652.
5. Babál P, Pecháňová O, Bernátová I: Long-term administration of D-NAME induces hemodynamic and structural changes in the cardiovascular system. Physiol Res, 2000, 49, 47–54.
6. Benter IF, Francis I, Cojocel C, Juggi JS, Yousif MH, Canatan H: Contribution of cytochrome P450 metabolites of arachidonic acid to hypertension and end-organ damage in spontaneously hypertensive rats treated with L-NAME. Auton Autacoid Pharmacol, 2005, 25, 143–154.
7. Benter IF, Yousif MH, Anim JT, Cojocel C, Diz DI: Angiotensin-(1-7) prevents development of severe hypertension and end-organ damage in spontaneously hypertensive rats treated with L-NAME. Am J Physiol Heart Circ Physiol, 2006, 290, H684–H691.
8. Bernátová I, Csizmadiová Z, Kopincová J, Púzserová A: Vascular function and nitric oxide production in chronic social-stress-exposed rats with various family history of hypertension. J Physiol Pharmacol, 2007, 58, 487–501.
9. Bernátová I, Kopincová J, Púzserová A, Janega P, Babál P: Chronic low-dose L-NAME treatment increases nitric oxide production and vasorelaxation in normotensive rats. Physiol Res, 2007, 56, Suppl 2, S17–S24.
10. Bernátová I, Pecháňová O, Babál P, Kyselá S, Štvrtina S, Andriantsitohaina R: Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. Am J Physiol, 2002, 282, H942–H948.
11. Bernátová I, Pecháňová O, Kristek F: Mechanism of structural remodelling of the rat aorta during long-term NG-nitro-L-arginine methyl ester treatment. Jpn J Pharmacol, 1999, 81, 99–106.
12. Bernátová I, Púzserová A, Kopincová J: Time- and dose-dependent modulation of nitric oxide production with L-NAME in borderline hypertensive rats. Physiol Res, 2010, 59, 2P–3P.
13. Bernátová, Pecháňová O, Šimko F: Effect of captopril in L-NAME-induced hypertension on the rat myocardium, aorta, brain and kidney. Exp Physiol, 1999, 84, 1095–1105.
14. Bevers LM, Braam B, Post JA, van Zonneveld AJ, Rabelink TJ, Koomans HA, Verhaar MC, Joles JA: Tetrahydrobiopterin, but not L-arginine, decreases NO synthase uncoupling in cells expressing high levels of endothelial NO synthase. Hypertension, 2006, 47, 87–94.
15. Chen HI, Hu CT: Endogenous nitric oxide on arterial hemodynamics: a comparison between normotensive and hypertensive rats. Am J Physiol, 1997, 273, 1816–1823.
16. Colasanti M, Persichini T, Menegazzi M, Mariotto S, Giordano E, Caldarera CM, Sogos V et al.: Induction of nitric oxide synthase mRNA expression. Suppression by exogenous nitric oxide. J Biol Chem, 1995, 270, 26731–26733.
17. Davis ME, Grumbach IM, Fukai T, Cutchins A, Harrison DG: Shear stress regulates endothelial nitric-oxide synthase promoter activity through nuclear factor κB binding. J Biol Chem, 2004, 279, 163–168.
18. Desai KM, Gopalakrishnan V, Hiebert LM, McNeill JR, Wilson TW: EDHF-mediated rapid restoration of hypotensive response to acetylcholine after chronic, but not acute, nitric oxide synthase inhibition in rats. Eur J Pharmacol, 2006, 546, 120–126.
19. Devlin AM, Brosnan MJ, Graham D, Morton JJ, McPhaden AR, McIntyre M, Hamilton CA et al.: Vascular smooth muscle cell polyploidy and cardiomyocyte hypertrophy due to chronic NOS inhibition in vivo. Am J Physiol, 1998, 274, H52–H59.
20. Ferreira-Melo SE, Yugar-Toledo JC, Coelho OR, De Luca IM, Tanus-Santos JE, Hyslop S, Irigoyen MC, Moreno H Jr: Sildenafil reduces cardiovascular remodeling associated with hypertensive cardiomyopathy in NOS inhibitor-treated rats. Eur J Pharmacol, 2006, 542, 141–147.
21. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature, 1980, 288, 373–376.

22. Gomez-Cabrera MC, Borrás C, Pallardó FV, Sastre J, Ji LL, Vina J: Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. J Physiol, 2005, 567, 113–120.
23. Grumbach IM, Chen W, Mertens SA, Harrison DG: A negative feedback mechanism involving nitric oxide and nuclear factor kappa-B modulates endothelial nitric oxide synthase transcription. J Mol Cell Cardiol, 2005, 39, 595–603.
24. Gur S, Kadowitz PJ, Gurkan L, Chandra S, Dewitt SY, Harbin A, Sikka SC et al.: Chronic inhibition of nitric-oxide synthase induces hypertension and erectile dysfunction in the rat that is not reversed by sildenafil. BJU Int, 2010, 106, 78–83.
25. Hayakawa H, Raij L: The link among nitric oxide synthase activity, endothelial function, and aortic and ventricular hypertrophy in hypertension. Hypertension, 1997, 29, 235–241.
26. Henrion D, Dowell FJ, Levy BI, Michel JB: In vitro alteration of aortic vascular reactivity in hypertension induced by chronic NG-nitro-L-arginine methyl ester. Hypertension, 1996, 28, 361–366.
27. Hobbs AJ, Higgs A, Moncada S: Inhibition of nitric oxide synthase as a potential therapeutic target. Annu Rev Pharmacol Toxicol, 1999, 39, 191–220.
28. Holécyová A, Török J, Bernátová I, Pecháňová O: Restriction of nitric oxide rather than elevated blood pressure is responsible for alterations of vascular responses in nitric oxide-deficient hypertension. Physiol Res, 1996, 45, 317–321.
29. Hu CT, Chang KC, Wu CY, Chen HI: Acute effects of nitric oxide blockade with L-NAME on arterial haemodynamics in the rat. Br J Pharmacol, 1997, 122, 1237–1243.
30. Hu W, Yang X, Zhe C, Zhang Q, Sun L, Cao K: Puerarin inhibits iNOS, COX-2 and CRP expression via suppression of NF-κB activation in LPS-induced RAW264.7 macrophage cells. Pharmacol Rep, 2011, 63, 781–789.
31. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G: Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA, 1987, 84, 9265–9269.
32. Jendeková L, Kojšová S, Andriantsitohaina R, Pecháňová O: The time-dependent effect of Provinols on brain NO synthase activity in L-NAME-induced hypertension. Physiol Res, 2006, 55, Suppl 1, S31–37.
33. Jendeková L, Kojšová S, Pecháňová O: The effect of apocynin on the prevention of borderline and spontaneous hypertension. J Hypertens, 2006, 24, Suppl 4, S342.
34. Jin ZG, Wong C, Wu J, Berk BC. Flow shear stress stimulates Gab1 tyrosine phosphorylation to mediate protein kinase B and endothelial nitric-oxide synthase activation in endothelial cells. J Biol Chem, 2005, 280, 12305–12309.
35. Jover B, Herizi A, Ventre F, Dupont M, Mimran A: Sodium and angiotensin in hypertension induced by long-term nitric oxide blockade. Hypertension, 1993, 21, 944–948.
36. Kopincová J: The effect of long-term low dose L-NAME treatment on cardiovascular system of rats with family history of hypertension (Slovak). Univezita Komenského, 2010, http://stella.uniba.sk/zkp-storage/dpg/dostupne/LF/2010/2010-LF-AqZyuH/
37. Kopincová J, Púzserová A, Bernátová I: Biochemical aspects of nitric oxide synthase feedback regulation by nitric oxide. Interdiscip Toxicol, 2011, 4, 63–68.
38. Kopincová J, Púzserová A, Bernátová I: Chronic low-dose L-NAME treatment effect on cardiovascular system of borderline hypertensive rats: feedback regulation? Neuro Endocrinol Lett, 2008, 29, 784–789.
39. Küng CF, Moreau P, Takase H, Lüscher TF: L-NAME hypertension alters endothelial and smooth muscle function in rat aorta. Prevention by trandolapril and verapamil. Hypertension, 1995, 26, 744–751.
40. Ledingham JM, Laverty R: Nitric oxide synthase inhibition with N^{ω}-nitro-L-arginine methyl ester affects blood pressure and cardiovascular structure in the genetically hypertensive rat strain. Clin Exp Pharmacol Physiol, 1997, 24, 433–435.
41. Lee JB, Ha JH, Lee JH, Kim JY, Park SJ, Joo Y: Prooxidant effect of angiotensin II in the brains of borderline-hypertensive rats. Clin Psychopharmacol Neurosci, 2003, 1, 51–58.
42. Luss H, DiSilvio M, Litton AL, Molina y Vedia L, Nussler AK, Billiar TR: Inhibition of nitric oxide synthesis enhances the expression of inducible nitric oxide synthase mRNA and protein in a model of chronic liver inflammation. Biochem Biophys Res Commun, 1994, 204, 635–640.
43. Łuszczki JJ, Jaskólska A, Dworzański W, Zółkowska D: 7-Nitroindazole, but not N^{G}-nitro-L-arginine, enhances the anticonvulsant activity of pregabalin in the mouse maximal electroshock-induced seizure model. Pharmacol Rep, 2011, 63, 169–175.
44. Matsubara BB, Matsubara LS, Zornoff LAM, Franco M, Janicki JS: Left ventricular adaptation to chronic pressure overload induced by inhibition of nitric oxide synthase in rats. Basic Res Cardiol, 1998, 93, 173–181.
45. Milstien S, Katusic Z: Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. Biochem Biophys Res Commun, 1999, 263, 681–684.
46. Oliveira MW, Minotto JB, de Oliveira MR, Zanotto-Filho A, Behr GA, Rocha RF, Moreira JC, Klamt F: Scavenging and antioxidant potential of physiological taurine concentrations against different reactive oxygen/nitrogen species. Pharmacol Rep, 2010, 62, 185–193.
47. Olzinski AR, McCafferty TA, Zhao SQ, Behm DJ, Eybye ME, Maniscalco K, Bentley R et al.: Hypertensive target organ damage is attenuated by a p38 MAPK inhibitor: role of systemic blood pressure and endothelial protection. Cardiovasc Res, 2005, 66, 170–178.
48. Orange SJ, Ledingham JM, Laverty R: Cardiovascular effects of chronic nitric oxide synthase inhibition in genetically hypertensive rats. Clin Exp Pharmacol Physiol, 2000, 27, 488–493.
49. Overend J, Martin W: Differential effects of nitric oxide synthase inhibitors on endothelium-dependent and nitrergic nerve-mediated vasodilatation in the bovine ciliary artery. Br J Pharmacol, 2007, 150, 488–493.

50. Palmer RMJ, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature, 1987, 327, 524–526.
51. Palmer RMJ, Moncada S: A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. Biochem Biophys Res Commun, 1989, 158, 348–352.
52. Palmer RMJ, Rees DD, Ashton DS, Moncada S: L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem Biophys Res Commun, 1988, 153, 1251–1256.
53. Park SK, Lin HL, Murphy S: Nitric oxide limits transcriptional induction of nitric oxide synthase in CNS glial cells. Biochem Biophys Res Commun, 1994, 201, 762–768.
54. Park SK, Lin HL, Murphy S: Nitric oxide regulates nitric oxide synthase-2 gene expression by inhibiting NF-κB binding to DNA. Biochem J, 1997, 322, 609–613.
55. Paulis L, Pechanova O, Zicha J, Barta A, Gardlik R, Celec P, Kunes J, Simko F: Melatonin interactions with blood pressure and vascular function during l-NAME-induced hypertension. J Pineal Res, 2010, 48, 102–108.
56. Paulis L, Pechanova O, Zicha J, Krajcirovicova K, Barta A, Pelouch V, Adamcova M, Simko F: Melatonin prevents fibrosis but not hypertrophy development in the left ventricle of N^G-nitro-L-arginine-methyl ester hypertensive rats. J Hypertens Suppl, 2009, 27, S11–S16.
57. Paulis L, Zicha J, Kuneš J, Hojná S, Behuliak M, Celec P, Kojšová S et al.: Regression of L-NAME-induced hypertension: the role of nitric oxide and endothelium-derived constricting factor. Hypertens Res, 2008, 31, 793–803.
58. Pecháňová O, Bernátová I, Pelouch V, Babál P: L-NAME-induced protein remodeling and fibrosis in the rat heart. Physiol Res, 1999, 48, 353–362.
59. Pecháňová O, Kojšová-Vranková S, Jendeková L: Ambivalent effect of chronic L-NAME treatment in the heart and brain: the role of nuclear factor-κB. J Mol Cell Cardiol, 2008, 44, 798–799.
60. Pechanova O, Matuskova J, Capikova D, Jendekova L, Paulis L, Simko F: Effect of spironolactone and captopril on nitric oxide and S-nitrosothiol formation in kidney of L-NAME-treated rats. Kidney Int, 2006, 70, 170–176.
61. Peng HB, Libby P, Liao JK: Induction and stabilization of IκBα by nitric oxide mediates inhibition of NF-κB. J Biol Chem, 1995, 270, 14214–14219.
62. Pourageaud F, Freslon JL: Endothelial and smooth muscle properties of coronary and mesenteric resistance arteries in spontaneously hypertensive rats compared to WKY rats. Fundam Clin Pharmacol, 1995, 9, 37–45.
63. Púzserová A, Csizmadiová Z, Bernátová I: Effect of blood pressure on L-NAME-sensitive component of vasorelaxation in adult rats. Physiol Res, 2007, 56, Suppl 2, S77–S84.
64. Rees DD, Palmer RM, Schulz R, Hodson HF, Moncada S: Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br J Pharmacol, 1990, 101, 746–752.
65. Rogers NE, Ignarro LJ: Constitutive nitric oxide synthase from cerebellum is reversibly inhibited by nitric oxide formed from L-arginine. Biochem Biophys Res Commun, 1992, 189, 242–249.
66. Sander M, Hansen PG, Victor RG: Sympathetically mediated hypertension caused by chronic inhibition of nitric oxide. Hypertension, 1995, 26, 691–695.
67. Sanders BJ, Lawler JE: The borderline hypertensive rat (BHR) as a model for environmentally-induced hypertension: a review and update. Neurosci Biobehav Rev, 1992, 16, 207–217.
68. Southan GJ, Szabó C: Selective pharmacological inhibition of distinct nitric oxide synthase isoforms. Biochem Pharmacol, 1996, 51, 383–394.
69. Sun ZJ, Zhang ZE: Historic perspectives and recent advances in major animal models of hypertension. Acta Pharmacol Sin, 2005, 26, 295–301.
70. Sventek P, Li JS, Grove K, Deschepper CF, Schiffrin EL: Vascular structure and expression of endothelin-1 gene in L-NAME-treated spontaneously hypertensive rats. Hypertension, 1996, 27, 49–55.
71. Sylvester FA: L-NAME-Induced Hypertension in the WKY and BHR Rat. Drake University, Des Moines, 1997.
72. Šimko F, Matúšková J, Lupták I, Krajčírovičová K, Kucharská J, Gvozdjaková A, Babál P, Pechanova O: Effect of simvastatin on remodelling of the left ventricle and aorta in L-NAME induced hypertension. Life Sci, 2004, 74, 1211–1224.
73. Takahashi T, Ono H, Ono Y, Ishimitsu T, Matsuoka H: Combination therapy with telmisartan and spironolactone alleviates L-NAME exacerbated nephrosclerosis with an increase in PPAR-γ and decrease in TGF-β1. Int Heart J, 2007, 48, 637–647.
74. Talarek S, Listos J, Fidecka S: Effect of nitric oxide synthase inhibitors on benzodiazepine withdrawal in mice and rats. Pharmacol Rep, 2011, 63, 680–689.
75. Török J: Participation of nitric oxide in different models of experimental hypertension. Physiol Res, 2008, 57, 813–825.
76. Török J, Holécyová A, Kyselá S, Bernátová I, Pecháňová O: Changes in reactivity of pulmonary and systemic arteries in chronic NO-deficient hypertension (Slovak). Kardiológia, 1998, 7, 30–36.
77. Tucker EJ, Ledingham JM, Zheng Y, Laverty R: Effects of chronic inhibition of nitric oxide synthase in the genetically hypertensive rat. Clin Exp Pharmacol Physiol, 2000, 27, 647–649.
78. Varagic J, Jerkic M, Jovovic D, Nastic-Miric D, Adanja-Grujic G, Markovic-Lipkovski J, Lackovic V et al.: Regional hemodynamics after chronic nitric oxide inhibition in spontaneously hypertensive rats. Am J Med Sci, 2000, 320, 171–176.
79. Vaziri ND, Ni Z, Oveisi F: Upregulation of renal and vascular nitric oxide synthase in young spontaneously hypertensive rats. Hypertension, 1998, 31, 1248–1254.
80. Vaziri ND, Ni Z, Oveisi F, Trnavsky-Hobbs DL: Effect of antioxidant therapy on blood pressure and NO synthase expression in hypertensive rats. Hypertension, 2000, 36, 957–964.
81. Vaziri ND, Wang XQ: cGMP-mediated negative-feedback regulation of endothelial nitric oxide synthase

expression by nitric oxide. Hypertension, 1999, 34, 1237–1241.
82. Vrankova S, Parohova J, Barta A, Janega P, Simko F, Pechanova O: Effect of nuclear factor kappa B inhibition on L-NAME-induced hypertension and cardiovascular remodelling. J Hypertens, 2010, 28, Suppl 1, S45–S49.
83. Wang YX, Poon CI, Pang CCY: In vitro and ex vivo inhibitory effects of L- and D-enantiomers of N^G-nitro--arginine on endothelium-dependent relaxation of rat aorta. J Pharmacol Exp Ther, 1993, 265, 112–119.
84. Wilcox CS: Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension? Am J Physiol Regul Integr Comp Physiol, 2005, 289, R913–R935.
85. Wuorela H, Arvola P, Kahonen M, Vapaatalo H, Porsti I: Arterial smooth muscle responses in adult and moderately aged spontaneously hypertensive rats. Pharmacol Toxicol, 1994, 74, 167–173.
86. Yamada SS, Sassaki AL, Fujihara CK, Malheiros DM, De Nucci G, Zatz R: Effect of salt intake and inhibitor dose on arterial hypertension and renal injury induced by chronic nitric oxide blockade. Hypertension, 1996, 27, 1165–1172.
87. Zanfolin M, Faro R, Araujo EG, Guaraldo AM, Antunes E, De Nucci G: Protective effects of BAY 41-2272 (sGC stimulator) on hypertension, heart, and cardiomyocyte hypertrophy induced by chronic L-NAME treatment in rats. J Cardiovasc Pharmacol, 2006, 47, 391–395.
88. Zhen J, Lu H, Wang XQ, Vaziri ND, Zhou XJ: Upregulation of endothelial and inducible nitric oxide synthase expression by reactive oxygen species. Am J Hypertens, 2008, 21, 28–34.

Received: October 24, 2011; **in the revised form:** January 27, 2012; **accepted:** February 14, 2012.