Physiol. Res. 49: 27-35, 2000

MINIREVIEW

Nitric Oxide-Compromised Hypertension: Facts and Enigmas

M. GEROVÁ

Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic

Received July 30, 1999
Accepted September 21, 1999

Summary

NO concentration in the femoral artery and femoral vein of anesthetized dogs was found to be 154.2±5.6 nM and 90.0±12 nM, respectively. Inhibition of NO synthase (NOS) slightly decreased the basal NO concentration in femoral artery from 154.2±5.6 to 137.2±3.3 nM. Acetylcholine-induced increase in NO concentration was slightly but still significantly attenuated, suggesting that very probably L-NAME did not inhibit all sources of nitric oxide (NO). Local NOS inhibition in the posterior hypothalamus dose-dependently increased systemic blood pressure (BP) in rats. Short-term general NOS inhibition in anesthetized dogs increased diastolic BP but not systolic BP. The heart rate after one-hour down-fluctuation returned to initial values. Proteosynthesis in the myocardium and both branches of the left coronary artery increased, but this was not supported by polyamines, since the activity of ornithine decarboxylase declined. Long-term general NOS inhibition elicited a sustained BP increase, a decrease in heart rate, cardiac hypertrophy and an increase in wall thickness of the coronary and carotid artery. The results indicate that NO deficiency itself plays a role in proteosynthesis and cardiac hypertrophy, in spite of relatively small increase in diastolic blood pressure and no change in systolic blood pressure, at least after an acute L-NAME administration. The hypotension response to acetylcholine and bradykinin studied in anesthetized NO-compromised rats, was unexpectedly enhanced. The elucidation of this paradoxical phenomenon will require further experiments.

Key words

NO level *in vivo* • Hypothalamus • Hypertension • L-NAME acute administration • L-NAME chronic administration • Polyamines

NO concentration measured *in vivo* in vessels close to endothelium

After Palmer *et al.* (1987, 1988) voiced the idea that nitric oxide is a by-product of the catalysis of arginine to citrulline induced by activation of the enzyme NO synthase, and that nitric oxide may underlie the endothelium-derived relaxation factor (EDRF) (Furchgott and Zawadzki 1980), only a few papers have reported values of NO measured directly, and if reported, then predominantly in cultures of endothelial cells or in vessels *in vitro*. Considering the lability of NO and its short half-life, our aim was to measure NO directly in the periendothelial area of vessels *in vivo*, namely its basal

Fax +420 2 293 894
E-mail physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

concentration and the concentration after intervening in nitric oxide production.

In our experiments on anesthetized dogs, blood flow was recorded in femoral artery. Using the porphyrinic biosensor (Malinski and Taha 1992) localized closely to the endothelium either in the femoral artery or in femoral vein, the respective values 154.2±5.6 and 90.0±12 nM of nitric oxide were found (Gerová *et al.* 1996a, 1998a).

Surprising results were obtained by interfering with the metabolic pathway of arginine → citrulline and NO production. Inhibition of endothelial NO synthase was induced by N^G-nitro-L-arginine-methyl ester (L-NAME 50 mg/kg) administered into the femoral artery *via* a multiperforated catheter introduced from the contralateral iliac artery. The following data were obtained: (i) Basal NO concentration, determined close to the endothelium of the femoral artery, declined from 154.2±5.6 nM to 137.2±3.3 nM (Gerová *et al.* 1998a). Kelm *et al.* (1999) used serum nitrite concentration as an indirect index of NO concentration and found a 30 % decrease after inhibition of NO synthase, i.e. also a relatively small decrease. (ii) Furthermore, the increase in NO concentration induced by acetylcholine (3-4 μg/kg/ml/min) was only mildly attenuated. In spite of NO synthase inhibition, acetylcholine brought about a significant increase in NO concentration by 125.3±8.3 nM. Bradykinin (30-40 μg/kg/ml/min) elevated the level of NO even more markedly, i.e. by 156.6±26.9 nM.

The blood flow in the femoral artery, concomitantly recorded by a Stattham flowmeter, reflected closely the fluctuation of NO concentrations in the periendothelial area of the femoral artery.

On the contrary, the experiments performed on isolated vessels *in vitro* showed that vasorelaxation responses to acetylcholine (ACh) and bradykinin (BK) were remarkably attenuated or almost absent after NO synthase inhibition (Rees *et al.* 1990, Kyselá and Török 1996, Holécyová *et al.* 1996).

It can be summarized that (i) a remarkably higher NO concentration was found in the periendothelial area of the femoral artery than in that of the femoral vein, and (ii) inhibition of NO synthase elicited only a slight decline in basal NO levels and a slight attenuation of the NO increment induced by acetylcholine and bradykinin. These results raised the question whether catalysis of arginine to citrulline is the only source of NO.

NO synthase inhibition underlies blood pressure increase

Two independent laboratories, Ribeiro *et al.* (1992) in Brazil and Baylis *et al.* (1992) in USA, found a sustained blood pressure elevation after a long-lasting inhibition of NO-synthase by administering L-NAME in drinking water to rats. In that year, the NO-deficient hypertension was introduced into the family of well-known experimental models of hypertension (Goldblatt renal hypertension, Okamoto spontaneous hypertension, DOCA-salt hypertension or Dahl salt-sensitive hypertension).

The most natural explanation of this hypertension was ascribed to the impaired relaxation and exaggerated readiness to amplify constriction of vessels, which was due to insufficient production of NO in endothelial cells. The endothelium is only one element of NO production determining the peripheral resistance *via* vascular smooth muscle tone. Constitutive NOS was also found in vascular myocytes (Bernhardt *et al.* 1991, Boulanger *et al.* 1998) and cardiac myocytes (Kelly *et al.* 1996). Consequently, continuous basal production of NO might be expected in the vessel wall and cardiac wall from all the given sources.

Constitutive NOS was also found in the neural structures relevant for cardiovascular control, i.e. in the CNS (cortex, hypothalamus, rostral ventrolateral medulla) and in the peripheral autonomic nervous system. Rodrigo *et al.* (1994) and Egberongbe *et al.* (1994) provided a map of NOS localization in the brain. Shapoval *et al.* (1991) demonstrated that alterations of NO concentration in the ventrolateral medulla induced immediate changes in BP. In the peripheral autonomic nervous system, Dun *et al.* (1993) detected NOS in the superior cervical ganglion, whereas Klimaschewski *et al.* (1992) and Ursell and Mayes (1995) disclosed it in intracardiac ganglia. NO affected the process of release and uptake of mediators (Addicks *et al.* 1994, Zanzinger *et al.* 1994). Even nitroxidergic nerves were described in the vessel wall (Toda and Okumura 1992).

A further substantial component involved in BP control, the kidney, is abundantly endowed with constitutive NOS – both endothelial and neuronal. The former was found in the vas afferens and efferens, glomeruli, tubuli, while neuronal NOS is particularly associated with the macula densa (Kone and Baylis 1997).

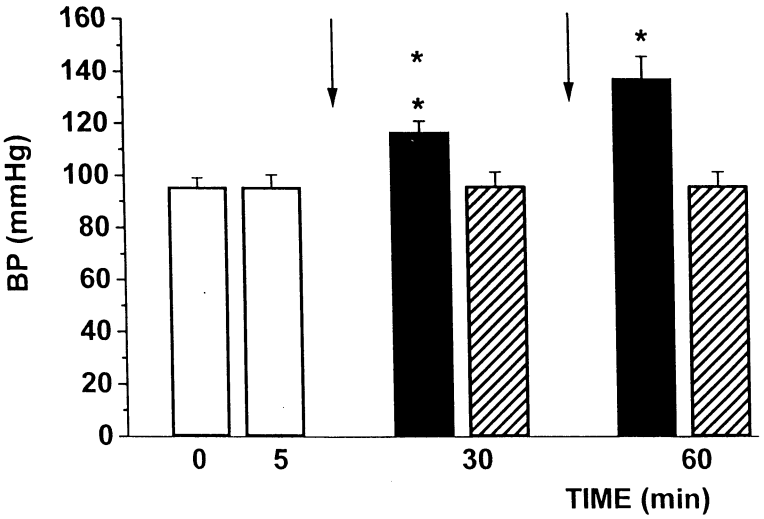

Fig. 1. *Open columns: mean blood pressure measured at 5 min of the steady-state period. Black columns: mean blood pressure 30 min after the first and second dose of L-NAME (0.3 mg/100 g b.w./3 µl, arrows) injected into the posterior hypothalamus of rats. Hatched columns: blood pressure of control rats 30 min after the first and second dose of 3 µl of artificial cerebrospinal fluid injected into the posterior hypothalamus.*

NO synthase inhibition in the area of posterior hypothalamus

The posterior hypothalamus is known to coordinate the known hemodynamic pattern, underlying Cannon's "fight or flight reaction". Using a stereotaxic apparatus and relevant parameters according to Paxinos and Watson (1982), a microcannula was inserted into the posterior hypothalamus and L-NAME was administered in microdoses (0.3 µg/100g b.w.) to inhibit NO synthase. The blood pressure increased and this elevation was further enhanced after repeating the microdose (Fig. 1). Control administration of the same dose of artificial cerebrospinal fluid did not increase blood pressure (Gerová *et al.* 1995).

Thus, BP increase may be induced by a slight imbalance in NO production in the distinct hypothalamic area, due to injury of the endothelium or due to simple hypoxia, because oxygen is a necessary prerequisite for catalysis of arginine to citrulline.

Generalized NO synthase inhibition

Early consequences of NO synthase inhibition

In the further series of experiments, an intervention into the arginine → citrulline pathway and NO production was performed in the whole organism. An immediate effect of NO synthase inhibition on blood pressure and heart rate in anesthetized dogs was recorded and proteosynthesis in the myocardium and epicardial coronary arteries was measured. The proteosynthesis was of interest because controversial data have been reported.

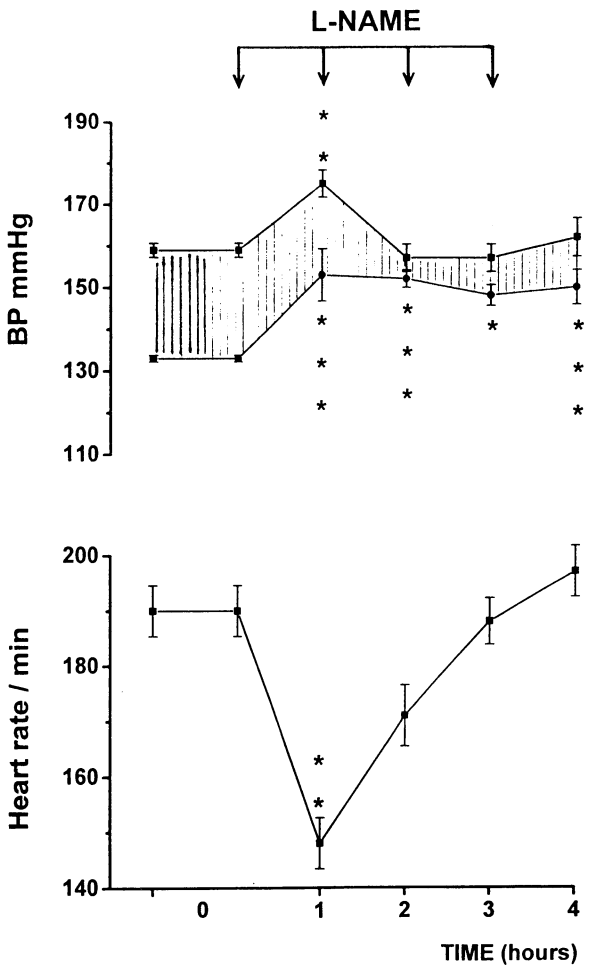

Fig. 2. *Systolic and diastolic blood pressure and heart rate of dogs before and one hour after the first, second, third and fourth dose of L-NAME (50 mg/kg b.w.).*

Although every increase in blood pressure, even a short-term one, is expected to increase proteosynthesis, neither Arnal *et al.* (1993) nor Banting *et al.* (1997) found cardiac hypertrophy in hypertensive rats with long-term inhibition of NOS.

In anesthetized dogs, L-NAME was administered i.v. in a dose of 50 mg/kg b.w. each hour in the course of 4 hours, i.e. a total of 200 mg/kg b.w. Figure 2 represents diastolic and systolic blood pressure before and during L-NAME administration. Diastolic blood pressure increased significantly and remained elevated till the end of the experiment. The course of changes in systolic BP had a rather unexpected pattern. After the first dose, systolic BP also increased, yet it then declined and did not differ significantly from the steady-state value. The pulse amplitude was extremely small, indicating a probable decline in stroke volume. The heart rate decreased after the first dose, and similarly as systolic BP, it returned to the steady-state value.

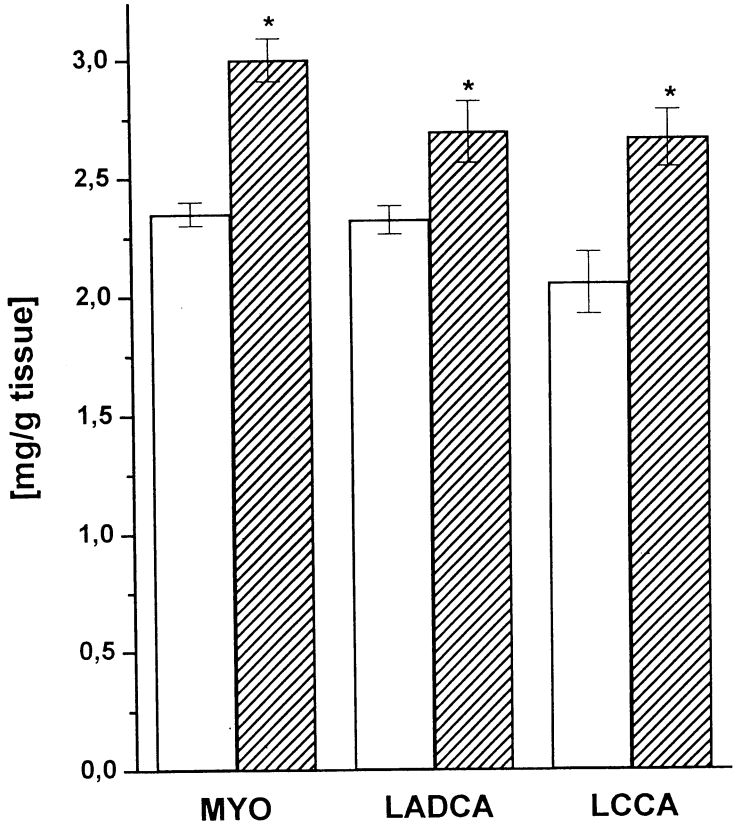

Fig. 3. *Total RNA content in the left ventricle myocardium (MYO), left descending coronary artery (LADCA) and left circumflex coronary artery (LCCA) of anesthetized dogs. Open columns: samples from control animals; hatched columns: samples from animals administered L-NAME (in a total dose of 200 mg/kg b.w.) for a period of 4 hours. (Reprinted with permission from Gerová et al. 1998b).*

However, the relatively small increase in diastolic pressure induced by L-NAME, which lasted 4 hours, was a stimulus strong enough to increase the proteosynthesis as indicated by the total RNA content in the left myocardium (Fig. 3). Similarly as in the myocardium, proteosynthesis increased in both the left descending coronary artery and left circumflex coronary artery (Fig. 3). The elevation of proteosynthesis was confirmed by ^{14}C-leucine incorporation into proteins of LV myocardium and both left coronary arteries (Gerová *et al.* 1998b). A remarkable difference emerged when comparing proteosynthesis in two different short-lasting episodes of blood pressure elevation: (i) compromise of NO production for a period of 4 hours, and (ii) stenosis of abdominal aorta for the same period (Gerová *et al.* 1996b, 1998b). During the BP elevation due to aortic stenosis, the different deformation rate of the left descending coronary artery and of the left circumflex coronary artery markedly affected proteosynthesis, yet only by an increase in the former. On the other hand, during the BP elevation due to compromised NO production, proteosynthesis increased in both epicardial coronary arteries, independently of the deformation rate.

Polyamines, which are the product of another metabolic pathway of arginine, represent a supporting mechanism for proteosynthesis and myocardial hypertrophy (Matsushita *et al.* 1972, Bartolome *et al.* 1980). The activity of arginase catalyzes arginine to ornithine and ornithine is catalyzed by ornithine decarboxylase (ODC) to polyamines. The activity of

ODC is considered to reflect the level of polyamines. Surprisingly, in these experiments with a 4-hour load of increased blood pressure, the ODC activity decreased (Fig. 4).

Short-term inhibition of NO synthase resulted in increased proteosynthesis in the LV myocardium and both main branches of the left coronary artery. Enhanced proteosynthesis was not supported by polyamines, and was present in spite of the relatively small changes in blood pressure and heart rate.

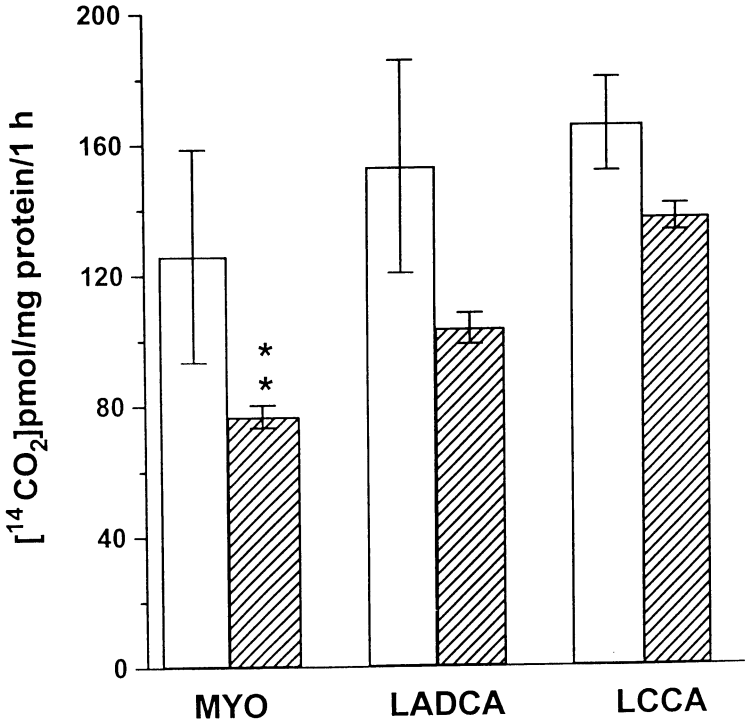

Fig. 4. *Ornithine decarboxylase activity in the left ventricle myocardium (MYO), left descending coronary artery (LADCA) and left circumflex coronary artery (LCCA) of anesthetized dogs. Open columns: samples from control animals; hatched columns: samples from animals administered L-NAME (in a total dose of 200 mg/kg b.w.) for a period of 4 hours. (Reprinted with permission from Gerová et al. 1998b).*

Consequences of long-term NO synthase inhibition

A long-term load of the cardiovascular system was induced by long-lasting inhibition of NO synthase. The rats were treated by L-NAME (50 mg/kg) in drinking water for 6 weeks. The blood pressure measured noninvasively each week was found to be continuously elevated, while the heart rate was declining.

An increase of *proteosynthesis* in the myocardium and aorta was already demonstrated after 4 weeks by Babál *et al.* (1997) and Pecháňová *et al.* (1999). In agreement with this, an increase in heart weight (1.10±0.03 g vs 1.32±0.09 g) and the heart weight/body weight ratio (2.1±0.04 vs 3.0±0.15) was found in these animals. As mentioned above, Arnal *et al.* (1993) and Banting *et al.* (1997) did not find cardiac hypertrophy. Using morphometry, we found an increase in crossectional area of myocytes, indicating myocyte hypertrophy, at least in subendocardial layers of the anterior wall of the left ventricle, from where the samples were taken (Sládek *et al.* 1996).

The *capillary domain* increased in the same range as the crossectional area of myocytes, indicating absence of angiogenesis (Sládek *et al.* 1996). Nevertheless, Arnal *et al.* (1993) and Babál *et al.* (1997) described ischemic areas with subsequent fibrosis in rat heart following a similar experimental intervention. Both the above processes are presumably occurring in the myocardium: the hypertrophy of myocytes might compensate the fibrotic areas.

The *sympathetic nervous system* has been supposed to operate as a tool of maintaining high BP (Sander *et al.* 1995). In cooperation with Doležel and Hartmannová, we studied the density of adrenergic nerve terminals in the myocardium, using the Falck fluorescence technique to visualize the terminals (Gerová *et al.* 1996c). A decrease in the number of adrenergic nerve terminals was found in the hypertrophic myocardium of rats with NO-deficient hypertension. The finding is in contrast to the opinion of Sander *et al.* (1995) , who suggested that sympathoadrenergic activity is increased. On the other hand, NO is considered a prerequisite for the development and normal function of the sympathetic nervous system (Peunova and Enikolopov 1995). In good concert with our results are the experiments of Scrogin *et al.* (1998) and Liu *et al.* (1998), who found no changes in plasma levels and a

decrease in the urinary excretion of noradrenaline and adrenaline. They concluded that the sympathetic nervous system was suppressed in L-NAME hypertension.

As far as the *conduit vessels* are concerned, the carotid artery was severely remodeled in NO-compromised hypertension (Delacretaz *et al.* 1994). We demonstrated that the wall thickness as well as the wall/diameter ratio were increased in the coronary and the carotid artery (Kristek *et al.* 1996, Kristek and Gerová 1996). With regard to the carotid and coronary artery, we have suggested that *resistance vessels*, decisive for BP elevation, are most probably also remodeled. Indeed, Li and Schiffrin (1994) demonstrated an increase in wall thickness of mesenterial arterioles of rats with NO-deficient hypertension.

Functional consequences of long-term inhibition of NO synthase

According to Folkow (1982) the resistance vessels in hypertension, either in human essential hypertension or in spontaneously hypertensive rats, are characterized by increased wall thickness and increased wall/diameter ratio. This is a reason why they are not able to dilate in the range as do the vessels from normotensives. Since remodeling of conduit and resistance vessels in NO compromised hypertension has unequivocally been proved in several laboratories, we addressed the question how the cardiovascular system functionally reflects the above morphological alterations.

According to *in vitro* experiments on vessels from animals with inhibited NO synthase (Rees *et al.* 1990, Kyselá and Török 1996, Holécyová *et al.* 1996), we expected the attenuation of hypotension elicited by acetylcholine. The response to acetylcholine and bradykinin was followed *in vivo*, in anaesthetized NO-compromised hypertensive rats (NOS inhibition lasting 6 weeks as mentioned above), and compared to control age-matched animals. Figure 5 demonstrates the extent of hypotension in rats to acetylcholine which was administered intravenously in three doses of 1, 5 and 10 μg. The ACh-induced hypotension in hypertensive rats was not only preserved but was even significantly enhanced, as compared to control animals. The same holds true for a completely different activator of NO synthase – bradykinin (100 μg/kg).

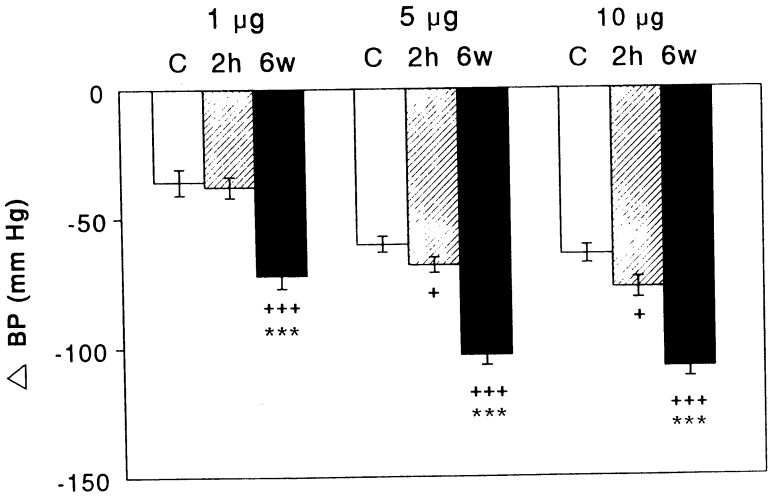

Fig. 5. *Blood pressure decrease induced by acetylcholine i.v. (1 μg, 5 μg, 10 μg/0.1 ml Krebs solution) in control rats (open columns), in rats 2 h after L-NAME had been applied (hatched columns), and in rats administered L-NAME (50 mg/kg b.w daily) continually for a period of six weeks (black columns).*

In these hypertensive rats, atropine totally abolished the acetylcholine hypotension, indicating that normally operating muscarinic receptors are involved. The bradykinin response was preserved after atropine.

The results of *in vivo* experiments differ significantly from *in vitro* studies on isolated vessels, in which the relaxation to acetylcholine was attenuated or even abolished completely after the inhibition of NO synthase (even when using L-NAME as inhibitor). Moreover, our results from *in vivo* experiments apparently contradict Folkow's idea (1982) that vessels remodeled due to hypertension are unable to relax to the extent seen in the controls. To solve this contradiction, further studies on the hemodynamic pattern (including cardiac output estimations) are necessary in NO-compromised hypertension. Schulze and Steiger, who discovered arginine at the end of the last century, had no idea that arginine plays such a basic and substantial role in cardiovascular control. In spite of the extensive studies of this topic during the last 20 years, basic data, which

should make it possible to solve many contradictory findings, are hitherto missing.

Acknowledgements

The author is grateful to Ms. Katarína Šoltésová, Anna Buzalková and Soňa Kyselá for assistance in preparing this manuscript. The study was supported by the VEGA Grant 2/4100/1999, and partially by Kapital a.s. Košice. This work was presented at the International Symposium "*Nitric Oxide: From Molecular Level to Clinical Application*" held in Bratislava, June 27-29, 1999 and published as an abstract in *Physiol Res* **48:** 30P, 1999.

References

ADDICKS K, BLOCH W, FEELISCH M: Nitric oxide modulates sympathetic neurotransmission at the prejunctional level. *Microscopy Res Tech* **29**: 161-168, 1994.

ARNAL JF, EL AMRANI AI, CHATELLIER G, MÉNARD J, MICHEL JB: Cardiac weight in hypertension induced by nitric oxide synthase blockade. *Hypertension* **22**: 380-387, 1993.

BABÁL P, PECHÁŇOVÁ O, BERNÁTOVÁ I, ŠTVRTINA S: Chronic inhibition of NO synthesis produces myocardial fibrosis and arterial media hyperplasia. *Histol Histopathol* **12**: 623-629, 1997.

BANTING JD, THOMPSON KE, FRIBERG P, ADAMS MA: Blunted cardiovascular growth induction during prolonged nitric oxide synthase blockade. *Hypertension* **30**: 416-421, 1997.

BARTOLOME J, HUGUENARD J, SLOTKIN TA: Role of ornithine decarboxylase in cardiac growth and hypertrophy. *Science* **210**: 793-794, 1980.

BAYLIS C, MITRUKA B, DENG A: Chronic blockade of nitric oxide synthases in the rat produces systemic hypertension and glomerular damage. *J Clin Invest* **90**: 278-281, 1992.

BERNHARDT J, TSCHUDI MR, DOHI Y, GUT I, URWYLER B, BÜHLER FR, LÜSCHER TF: Release of nitric oxide from human vascular smooth muscle cells. *Biochem Biophys Res Commun* **180**: 907-912, 1991.

BOULANGER CM, HEYMES CH, BENESSIANO J, GESKE RS, LÉVY BI, VANHOUTTE PM: Neuronal nitric oxide synthase is expressed in rat vascular smooth muscle cells: activation by angiotensin II in hypertension. *Circ Res* **83**: 1271-1278, 1998.

DELACRETAZ E, HAYOZ D, OSTERHELD MC, GENTON CY, BRUNNER HR, WEBER B: Long-term nitric oxide synthase inhibition and distensibility of carotid artery in infant rats. *Hypertension* **23**: 967-970, 1994.

DUN NJ, DUN SL, WU SY, FÖRSTERMANN U: Nitric oxide synthase immunoreactivity in rat superior cervical ganglia and adrenal glands. *Neurosci Lett* **158**: 51-54, 1993.

EGBERONGBE YI, GENTLEMAN SM, FALKAI P, BOGERTS B, POLAK JM, ROBERTS GW: The distribution of nitric oxide synthase immunoreactivity in the human brain. *Neuroscience* **59**: 561-578, 1994.

FOLKOW B: Physiological aspects of primary hypertension. *Physiol Rev* **62**: 347-504, 1982.

FURCHGOTT RF, ZAWADZKI JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288:** 373-376, 1980.

GEROVÁ M: NO compromised hypertension: facts and enigmas. *Physiol Res* **48:** 30P, 1999.

GEROVÁ M, MAŠÁNOVÁ C, PAVLÁSEK J: Inhibition of NO synthase in the posterior hypothalamus increases blood pressure in the rat. *Physiol Res* **44**: 131-134, 1995.

GEROVÁ M, MESAROŠ Š, KITTOVÁ M, HATRIK Š, KRISTEK F, MALINSKI T: Nitric oxide in the periendothelial area of femoral vein of the dog assessed in vivo by a porphyrinic sensor. *Physiol Res* **45**: 285-289, 1996a.

GEROVÁ M, PECHÁŇOVÁ O, STOEV V, KITTOVÁ M, BERNÁTOVÁ I, BARTA E: Early changes in protein synthesis in epicardial coronary artery of pressure-overloaded heart. *Am J Physiol* **270**: H685-H691, 1996b.

GEROVÁ M, HARTMANNOVÁ B, DOLEŽEL S, JEŽEK L: Long-term inhibition of NO synthase induces cardiac hypertrophy with a decrease in adrenergic innervation. *Physiol Res* **45**: 339-344, 1996c.

GEROVÁ M, MESAROŠ Š, KRISTEK F, KITTOVÁ M, MALINSKI T: NO concentration in the periendothelial area of the femoral artery of the dog measured in vivo. *Physiol Res* **47**: 169-175, 1998a.

GEROVÁ M, PECHÁŇOVÁ O, BERNÁTOVÁ I, KITTOVÁ M, JURÁNI M: Early changes of protein synthesis in myocardium and coronary arteries induced by NO synthase inhibition. *Physiol Res* **47**: 405-412, 1998b.

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* **45**: 317-321, 1996.

KELLY RA, BALLIGAND JL, SMITH TW: Nitric oxide and cardiac function. *Circ Res* **79**: 363-380, 1996.

KELM M, PREIK-STEINHOFF H, PREIK M, STRAUER BE: Serum nitrite sensitively reflects endothelial NO formation in human forearm vasculature: evidence for biochemical assessment of the endothelial L-arginine-NO pathway. *Cardiovasc Res* **41**: 765-772, 1999.

KLIMASCHEWSKI L, KUMMER W, MAYER B, COURAUD JY, PREISSLER U, PHILIPPIN B, HEYM C: Nitric oxide synthase in cardiac nerve fibers and neurons of rat and guinea pig heart. *Circ Res* **71**:1533-1537, 1992.

KONE BC, BAYLIS C: Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. *Am J Physiol* **272**: F561-F578, 1997.

KRISTEK F, GEROVÁ M: Long-term NO synthase inhibition affects heart weight and geometry of coronary and carotid arteries. *Physiol Res* **45:** 361-367, 1996.

KRISTEK F, GEROVÁ M, DEVÁT L, VARGA I: Remodeling of septal branch of coronary artery and carotid artery in L-NAME treated rats. *Physiol Res* **45**: 329-333, 1996

KYSELÁ S, TÖRÖK J: Histamine H_1-receptor antagonists do not prevent the appearance of endothelium-dependent relaxation to acetylcholine in rat pulmonary artery. *Physiol Res* **45**: 245-350, 1996.

LI JS, SCHIFFRIN EL: Resistance artery structure and neuroeffector mechanisms in hypertension induced by inhibition of nitric oxide synthase. *Am J Hypertens* 7: 996-1004, 1994.

LIU Y, TSUCHIHASHI T, KAGIYAMA S, MATSUMURA K, ABE I, FUJISHIMA M: Central and peripheral mechanisms involved in hypertension induced by chronic inhibition of nitric oxide synthase in rats. *J Hypertens* **16**: 1165-1173, 1998.

MALINSKI T, TAHA Z: Nitric oxide release from a single cell measured in situ by a porphyrinic-based microsensor. *Nature* **358**: 676-678, 1992.

MATSUSHITA S, SOGANI RK, RABEN MS: Ornithine decarboxylase in cardiac hypertrophy in the rat. *Circ Res* **31**: 699-709, 1972.

PALMER RMJ, FERRIGE AG, MONCADA S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327**: 524-526, 1987.

PALMER RMJ, ASHTON DS, MONCADA S: Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333**: 664-666, 1988.

PAXINOS G, WATSON C (eds): *The Rat Brain.* Academic Press, London, 1982.

PECHÁŇOVÁ O, BERNÁTOVÁ I, PELOUCH V, BABÁL P: L-NAME induced protein remodeling and fibrosis in the rat heart. *Physiol Res* **48**: 353-362, 1999.

PEUNOVA N, ENIKOLOPOV G: Nitric oxide triggers a switch to growth arrest during differentiation of neuronal cells. *Nature* **375**: 68-73, 1995.

REES DD, PALMER RMJ, SCHULZ R, HODSON HF, MONCADA S: Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br J Pharmacol* **101**: 746-752, 1990.

RIBEIRO MO, ANTUNES E, DE NUCCI G, LOVISOLO SM, ZATZ R: Chronic inhibition of nitric oxide synthesis: a new model of arterial hypertension. *Hypertension* **20**: 298-303, 1992.

RODRIGO J, SPRINGALL DR, UTTENTHAL O, BENTURA ML, ABADIA-MOLINA F, RIVEROS-MORENO V, MARTINEZ-MURILLO R, POLAK JM, MONCADA S: Localization of nitric oxide synthase in the adult rat brain. *Phil Trans R Soc Lond B* **345**: 175-221, 1994.

SANDER M, HANSEN PG, VICTOR RG: Sympathetically mediated hypertension caused by chronic inhibition of nitric oxide. *Hypertension* **26**: 691-695, 1995.

SCROGIN KE, HATTON DC, CHI Y, LUFT FC: Chronic nitric oxide inhibition with L-NAME: effects on autonomic control of the cardiovascular system. *Am J Physiol* **274**: R367-R374, 1998.

SHAPOVAL LN, SAGACH VF, POBEGAILO LS: Nitric oxide influences ventrolateral medullary mechanisms of vasomotor control in the cat. *Neurosci Lett* **132**: 47-50, 1991.

SLÁDEK T, GEROVÁ M, ZNOJIL V, DEVÁT L: Morphometric characteristics of cardiac hypertrophy induced by long-term inhibition of NO synthase. *Physiol Res* **45**: 335-338, 1996.

TODA N, OKAMURA T: Regulation by nitroxidergic nerve of arterial tone. *News Physiol Sci* **7**: 148-152, 1992.

URSELL PC, MAYES M: Anatomic distribution of nitric oxide synthase in the heart. *Int J Cardiol* **50**: 217-223, 1995.

ZANZINGER J, CZACHURSKI J, SELLER H: Nitric oxide in the ventrolateral medulla regulates sympathetic responses to systemic hypoxia in pigs. *Am J Physiol* **275**: R33-R39, 1998.

Reprint requests

M. Gerová, MD, DSc, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Sienkewiczova 1, 813 71 Bratislava, Slovak Republic, Fax +421-7-368 516, e-mail: gerova@unpf.savba.sk.