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MINIREVIEW

The Role of Nitric Oxide in the Maintenance of Vasoactive Balance

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Summary

Endothelial dysfunction may be considered as the interstage between risk factors and cardiovascular pathology. An imbalance between the production of vasorelaxing and vasoconstricting factors plays a decisive role in the development of hypertension, atherosclerosis and target organ damage. Except vasorelaxing and antiproliferative properties *per se*, nitric oxide participates in antagonizing vasoconstrictive and growth promoting effects of angiotensin II, endothelins and reactive oxygen species. Angiotensin II is a potent activator of NAD(P)H oxidase contributing to the production of reactive oxygen species. Numerous signaling pathways activated in response to angiotensin II and endothelin-1 are mediated through the increased level of oxidative stress, which seems to be in casual relation to a number of cardiovascular disturbances including hypertension. With respect to the oxidative stress, the NO molecule seems to be of ambivalent nature. On the one hand, NO is able to reduce generation of reactive oxygen species by inhibiting association of NAD(P)H oxidase subunits. On the other hand, when excessively produced, NO reacts with superoxides resulting in the formation of peroxynitrite, which is a free radical deteriorating endothelial function. The balance between vasorelaxing and vasoconstricting substances appears to be the principal issue for the physiological functioning of the vascular bed.

Key words

Endothelial dysfunction • Hypertension • Nitric oxide • Angiotensin II • Endothelins • Reactive oxygen species

Introduction

An imbalance in the production of angiotensin II, endothelins, reactive oxygen species on the one hand and nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) on the other hand plays an important pathogenetic role in hypertension and hypertensive end-organ injury (Noll *et al*. 1997, Šimko and Šimko 1999, Schiffrin 2002, Púzserová *et al*. 2007). Angiotensin II (Ang II) stimulates nicotinamide adenine

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dinucleotide phosphate (NADPH) oxidase in the endothelium, smooth muscle cells, and the adventitia of blood vessels to generate reactive oxygen species, supporting endothelial dysfunction, vascular remodeling, and inflammation. Upregulation of endothelin-1, growth factors, adhesion molecules, nuclear factor–κB (NF-κB), and other inflammatory mediators, as well as increased breakdown of nitric oxide and uncoupling of nitric oxide synthase may be involved in the progression of vascular pathologies (Schiffrin 2002, Hamilton *et al.* 2002).

Hypertension is accompanied with functional and structural alterations in the number of systems and tissues. Cardiac hypertrophy, fibrosis enlargement, smooth muscle cell hypertrophy/hyperplasia, vascular remodeling, endothelial dysfunction, atherosclerosis and microalbuminuria rank among the most important changes (Pecháňová *et al.* 1999, 2006b, Bernátová *et al.* 2006; Cebová *et al.* 2006, Čačányiová *et al.* 2006) resulting ultimately in heart failure, renal insufficiency, myocardial infarction or stroke (Török *et al.* 2006, Šimko 2007) (Fig. 1). In this context, predominantly those antihypertensive agents are considered that beside blood pressure lowering also restore the humoral balance within the vascular wall.

Antihypertensive action may be exerted both on the level of resistance vessels and in the central nervous system, depressing the increased sympathetic tone which is one of the most powerful tool for blood pressure control. Similarly, nitric oxide does not play a role only as a peripheral relaxing factor but it is also involved in the central regulation of sympathetic tone (Kuneš *et al.* 2004, Zicha *et al.* 2006a). Thus, the balance between vasorelaxing and vasoconstricting agents in vasomotor centers seems to be crucial for the control of the sympathetic tone.

The aim of this review is to analyze the role of endothelium and nitric oxide in the maintenance of vasoactive balance under pathophysiological circumstances, hypertension particularly.

Endothelium

The endothelium serves not just as a barrier of the transvascular diffusion but is the largest endocrine organ in the body. Its autocrine and paracrine actions play a critical role in the regulation of blood flow, coagulation, leukocyte adhesion, and vascular smooth muscle cell growth. Furchgott and Zawadzki (1980) demonstrated that the relaxation of vascular smooth muscle cells in response to acetylcholine is dependent on the integrity of the endothelium. Endothelium-derived relaxing factor was identified as a free radical gas – nitric oxide (NO) (Palmer *et al.* 1987, Ignarro *et al.* 1987). NO generation by endothelial cells is in principle constitutive but may be stimulated by a variety of compounds, including acetylcholine, angiotensin II, bradykinin and many others (Lüscher and Vanhoutte 1988). NO is not the sole endothelium-derived vasodilator. Prostacyclin (PGI_2), another endothelium-dependent vasodilator, relaxes the underlying smooth muscle cells through activation of adenylate cyclase and subsequent generation of cAMP. Constitutively released PGI_2 (Moncada *et al.* 1976) appears to be involved in the regulation of resting vascular tone. PGI_2 is released in higher amount in response to ligand binding on the cell surface such as thrombin, arachidonic acid, histamine, or serotonin. Endothelium also generates a hyperpolarizing factor, which is suspected to be an arachidonic acid metabolite produced by cytochrome P450 (Komori and Vanhoutte 1990). Within the endothelium, the synthesis and degradation of adenine nucleotides takes place. These purines can influence vascular tone and platelet aggregation through variable purinoceptors. Adenosine may serve as a vasodilator and potent inhibitor of platelet aggregation through stimulation of adenylate cyclase (Burnstock 1990, Pecháňová and Babál 1993).

Under some pathophysiological circumstances, e.g. in atherosclerosis or hypertension, endothelium-derived vasoconstricting factors can be released and contribute to the paradoxical vasoconstrictor effects. Apart from the peptides endothelin and angiotensin II, other endothelium-derived vasoconstricting agents such as superoxide anions, vasoconstrictor prostaglandins, and thromboxane A_2 have been postulated (Vanhoutte *et al.* 2005).

Nitric oxide

The discovery of endothelium-derived relaxing factor, nitric oxide, allowed formulation of a novel concept in the pathogenesis of hypertension involving a crucial role of endothelium and nitric oxide (Furchgott and Zawadzki 1980). NO is generated by NO synthases, a family of enzymes that convert the amino acid L-arginine to L-citrulline and NO. All NOS isoforms are homodimeric enzymes that require the same substrate (L-arginine), cosubstrates (molecular oxygen, NADPH) and cofactors (FMN, FAD, tetrahydrobiopterin, heme)

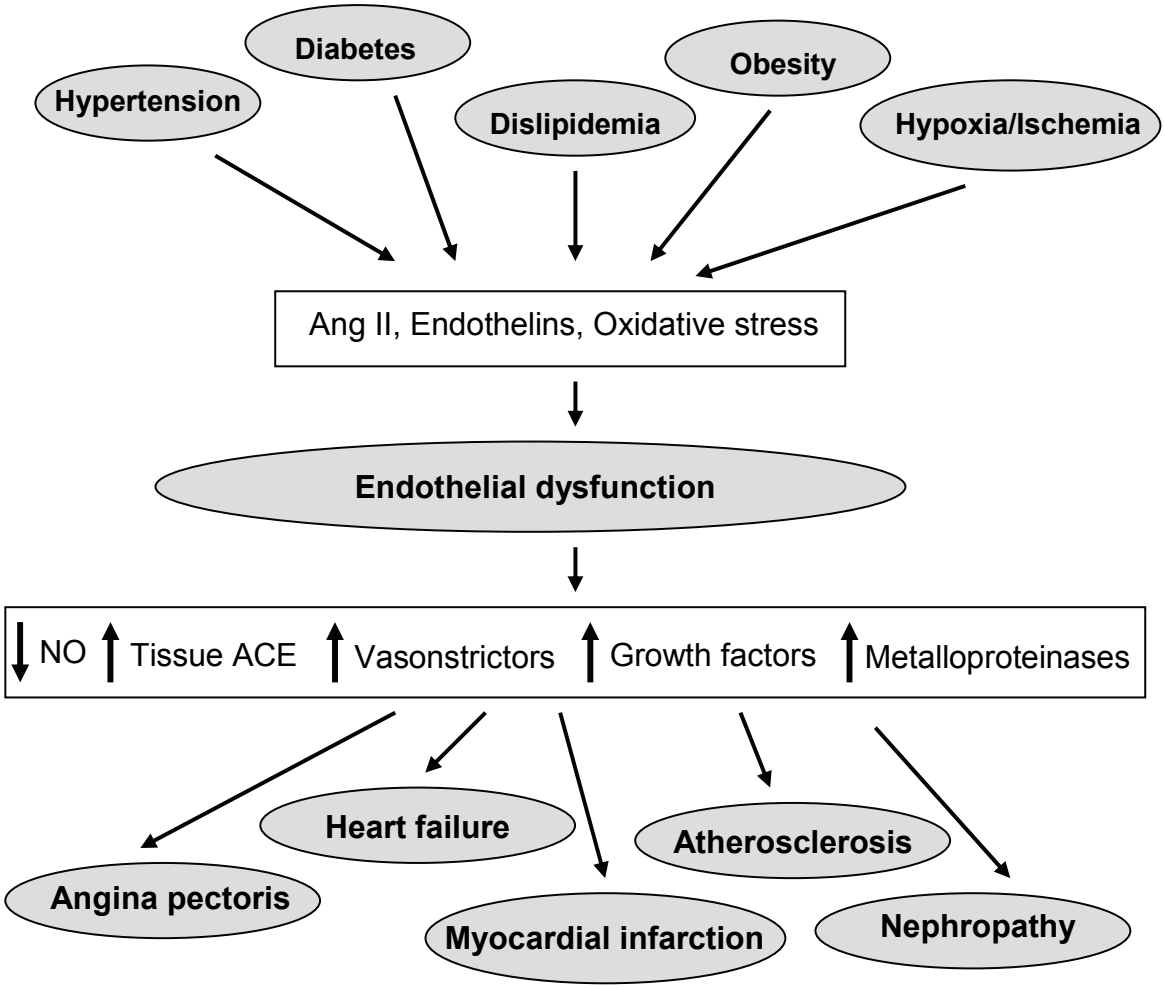

Fig. 1. Risk factors of cardiovascular diseases are associated with increased angiotensin II and endothelin production as well as with oxidative stress leading to endothelial dysfunction. Endothelial dysfunction can result in vascular abnormalities leading to the cardiovascular morbidity and kidney impairment. NO - nitric oxide; Ang II – angiotensin II, ACE - angiotensin converting enzyme

(Stuehr 1999). Four NOS isoforms have been described. They differ with respect to the main mode of regulation, the tissue expression pattern and the average amount of NO produced (Bernátová and Pecháňová 1998, Ghosh and Salerno 2003).

Endothelial NOS expressed in endothelial cells is the predominant NOS isoform in the vessel wall. Receptor-mediated agonist stimulation (e.g. bradykinin, acetylcholine, thrombin, histamine) leads to rapid enzyme activation by depalmitoylation, binding to calmodulin/calcium, displacement of caveolin and release from the plasma membrane (Govers and Rabelink 2001). In addition, shear stress is also an important modulator of eNOS activity. Endothelial NOS activity is also regulated at the transcriptional level because VEGF, insulin, bFGF increase eNOS expression while hypoxia and oxidized LDL decrease it (Sase and Michel 1997, Maxwell 2002). NO activates guanylate cyclase by binding to the heme moiety of this enzyme. Guanylate cyclase catalyzes the conversion of guanosine triphosphate (GTP) to cGMP, which in turn activates cGMP-dependent protein kinase. This kinase phosphorylates phospholamban, a regulatory protein for the Ca^{2+}-adenosine triphosphatase (ATPase) in the sarcoplasmic reticulum. The Ca^{2+}-ATPase decreases intracellular calcium concentration leading to smooth muscle relaxation (Sanders *et al.* 1995).

Except the vasorelaxing and antiproliferative properties *per se*, nitric oxide plays an important role in antagonizing the effects of Ang II, endothelins and reactive oxygen species.

Angiotensin II

Angiotensin II is a vasoconstrictor with the proliferative effect, involved in the regulation of salt and water homeostasis and pathological remodeling of the heart and vessels (Šimko and Šimko 1999). Ang II is a potent activator of NAD(P)H oxidase contributing to the

production of reactive oxygen species which participate in variable pathologies within the circulation (Šimko and Šimko 1999, Hitomi *et al*. 2007).

NO antagonizes the effects of Ang II on vascular tone, cell growth, and renal sodium excretion, and also down-regulates the synthesis of ACE and Ang II type 1 receptors (Zhou *et al.* 2004). It has been shown that treatment with ACE inhibitor captopril prevents or reduces L-NAME-induced increase of blood pressure and heart hypertrophy (Bernátová *et al.* 1996, Pecháňová *et al.* 1997). Animals receiving simultaneously L-NAME and ramipril were also protected against development of hypertension and myocardial hypertrophy as well as against the deterioration of glomerular filtration rate and renal plasma flow (Hropot *et al*. 1994). Enalapril also inhibited development of both arterial hypertension and left ventricular hypertrophy in NO-deficient hypertension but failed to prevent ischemic myocardial lesions. This suggests that the renin-angiotensin system (RAS) plays a major role in the development of hypertension and cardiac hypertrophy, but its participation in ischemia-induced myocardial alterations is less probable in NO-deficient hypertension (Moreno *et al*. 1995).

It was shown previously that ACE inhibition upregulates eNOS expression. The mechanism of this upregulation is still unclear. However, it is conceivable that ACE inhibitor-induced accumulation of endogenous kinins mediates this effect (Morawietz *et al.* 2006). In L-NAME treated animals, the increased expression as well as activation of eNOS are masked by competitive NO synthase inhibitor. These results are in good agreement with the finding that captopril completely prevented development of hypertension and left ventricular hypertrophy due to the L-NAME treatment, but without affecting NO synthase inhibition (Pecháňová *et al.* 1997, Bernátová *et al.* 1999, Šimko *et al.* 2003). Captopril and enalapril also prevented blood pressure rise in young spontaneously hypertensive rats. Captopril, probably due to the antioxidant role of its thiol group, had more effective hypotensive effect than enalapril (Pecháňová 2007).

Ang II type 1 receptor blocker, losartan, prevented the development of L-NAME-induced hypertension and impairment of vascular relaxation to nitroprusside, isoprenaline, and cromakalim, vasodilators acting *via* the formation of NO, activation of beta-adrenoceptors and opening of K^+ channels, respectively. Thus, losartan was able to improve both endothelium-dependent and -independent vascular relaxation. Hyperpolarization of smooth muscle cells, increased sensitivity to NO, and decreased oxidative stress in the vascular wall might participate on the protective effect of losartan (Kalliovalkama *et al*. 1999, Kitamoto *et al*. 2000).

Endothelins

The endothelin family consists of three structurally related peptides, ET-1, ET-2, and ET-3 (Kedzierski and Yanagisawa 2001). In the vasculature, the proendothelin may be released from the non-luminal surface of the endothelial cells and converted extracellularly to mature endothelin by membrane-bound endothelin-converting enzymes, which are neutral metalloproteinases. Endothelin does not appear to be stored in endothelial cells, but is synthesized *de novo* in response to several substances (thrombin, angiotensin II, cytokines) or physical stimuli (shear stress, hypoxia). Endothelin is a potent vasoconstricting agent with long-lasting effects (Rubanyi and Botelho 1991).

There are numerous interactions between some of the vasoactive agents released from the endothelium. Many factors that stimulate endothelin synthesis, (e.g. thrombin, Ang II), also cause the release of vasodilators such as PGI_2 and/or NO, which oppose the vasoconstricting action of endothelin. ET-1 also stimulates mitogenic activity on smooth muscle cells while NO and PGI_2 inhibit this proliferative effect (Alberts *et al.* 1994).

It seems that the vascular reactions are the result of a complex interaction of many vasoactive pathways. The relative importance of these actions may vary in dependence to vascular beds, animal species or underlying pathological processes.

Reactive oxygen species

Reactive oxygen species (ROS) participate on physiological reactions by mediation of signal transduction. On the other hand, the excessive or inappropriate production of ROS may exert deleterious effects on the cardiovascular system resulting in the occurrence of hypertension, atherosclerosis and their consequences (Touyz and Schiffrin 2004). Most important radicals are superoxide anion (O_2^-), hydroxyl radical ($^{\cdot}OH$), and the reactive nitrogen species – nitric oxide and peroxynitrite ($ONOO^-$). A number of factors supporting the elevation of blood pressure such as Ang II,

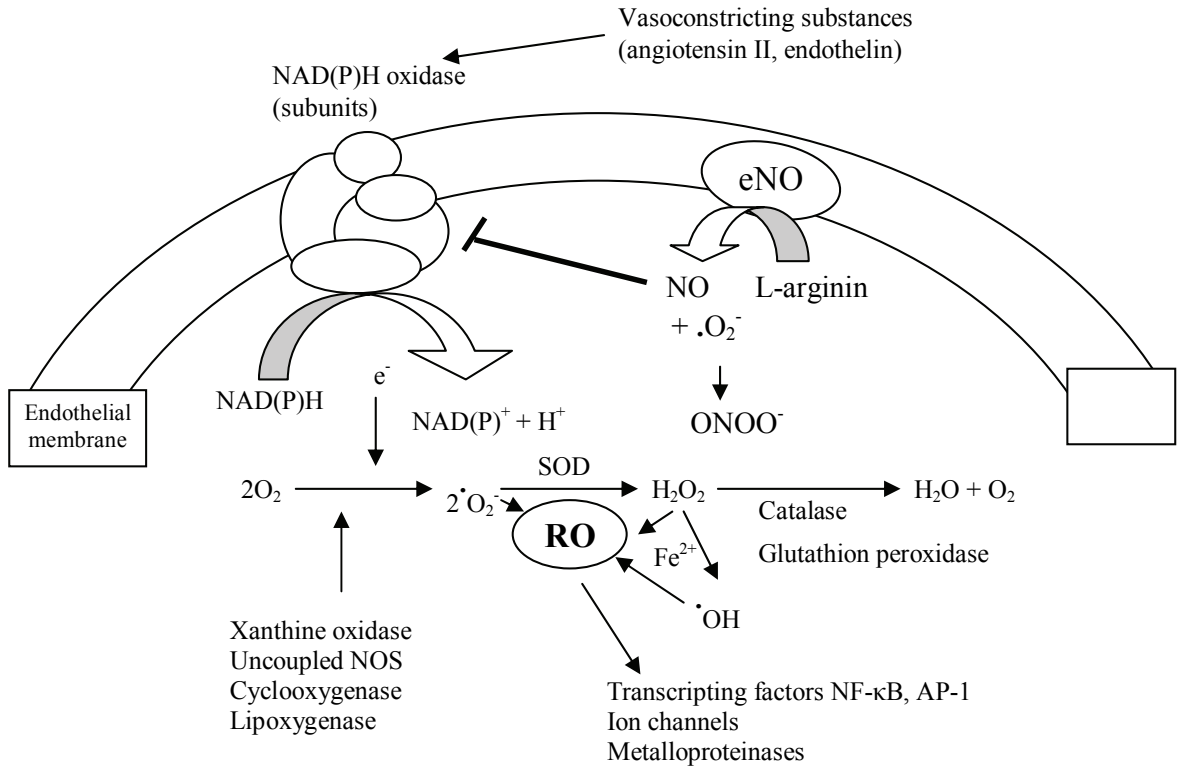

Fig. 2. Reactive oxygen species (ROS) generation in the cell. Vasoconstricting substances increase ROS production through NAD(P)H oxidase, xanthine oxidase, uncoupled NO synthase, cyclooxygenase, lipoxygenase and mitochondrial respiratory chain enzymes. ROS generation activates nuclear factor NF-κB and activator protein 1 (AP-1), metalloproteinases and modulates intracellular Ca^{2+} concentration through ion channels alterations. Enzymatic systems, superoxide dismutase (SOD), catalase and glutathione peroxidase, are active in the defense against ROS by forming H_2O_2 and H_2O. Endothelial NO synthase (eNOS) produces nitric oxide (NO) which prevents association of NAD(P)H oxidase subunits. Concurrently, NO may, however, react with superoxides by forming peroxynitrites ($ONOO^-$) with amplifying effect on endothelial dysfunction.

ET-1 or aldosterone stimulate ROS production through the activation of NAD(P)H oxidase, xanthine oxidase, lipoxygenase, uncoupled NO synthase, and mitochondrial respiratory chain enzymes. With respect to arterial hypertension development, NAD(P)H oxidase seems to be the main enzyme responsible for superoxide production (Lassegue and Griendling 2004) (Fig. 2).

Treatment with antioxidants was shown to decrease blood pressure, improve vascular function and structure and ameliorate target-organ damage in experimental as well as human hypertension (Lassegue and Griendling 2004, Púzserová *et al*. 2006). Antioxidant compounds, such as glutathione, vitamin C, vitamin E and uric acid provide non-enzymatic protection against oxidative stress. Enzymatic systems represented by superoxide dismutase (SOD), catalase and glutathione peroxidase are active in the defense against ROS by forming H_2O_2 and H_2O. Finally, drugs inhibiting the neurohumoral activation also reduce the oxidative stress (Touyz and Schiffrin 2004).

In several models of animal hypertension blood pressure was reduced and vascular remodeling inhibited by consuming a diet rich in vitamin C or E (Chen *et al*. 2001). Moreover, SOD mimetic tempol, decreased blood pressure, vascular hypertrophy and improved endothelium-dependent relaxation (Chen *et al*. 2001, Kawada *et al*. 2002). Similarly, overexpression of SOD and catalase reduced blood pressure, improved availability of NO and endothelium-dependent relaxation in different models of hypertension (Chu *et al*. 2003). Apocynin, NAD(P)H oxidase inhibitor also prevented blood pressure elevation and cardiovascular hypertrophy in aldosterone-infused and spontaneously hypertensive rats (Park *et al*. 2004, Kojšová *et al*. 2006).

Nitric oxide and maintenance of vasoactive balance

About 20 years have passed since the discovery of NO. During this relatively short time period, our knowledge on the role of endothelium and nitric oxide in cardiovascular diseases have tremendously increased. It is generally admitted that the normal production of NO with its vasodilative, antiaggregative and antiproliferative

action plays a crucial role in the maintenance of the physiologic conditions within the cardiovascular system.

L-arginine, a substrate for NO synthase, seems to be promising in preserving NO formation. However, L-arginine failed to prevent blood pressure increase and left ventricle remodeling due to chronic L-NAME treatment (Šimko *et al.* 2005). Some other effects of L-NAME, besides blood pressure increase and NO deficiency, could participate in this lack of L-arginine protection. It has been demonstrated that L-NAME inhibits L-arginine transport to the caveolae containing NO synthase (Maxwell 2002). Moreover, L-NAME increased the activity of nuclear factor-κB, which may participate in cardiovascular remodeling independently of the blood pressure increase (Pecháňová *et al.* 2004a).

As previously mentioned, ACE inhibitor captopril completely prevented NO-deficient hypertension, yet without improving NO synthase activity. It was suggested that both inhibition of Ang II formation and enhanced production of PGI_2 caused by increased bradykinin level may be responsible for observed protective effect of captopril. In the regression experiment, three weeks of spontaneous recovery failed to reverse hypertrophy developed by L-NAME treatment, whereas captopril treatment reversed both hypertension and left ventricular hypertrophy (Bernátová *et al*. 2000). Captopril also reduced blood pressure, improved aortic relaxation and reduced heart and aortic remodeling in the hereditary hypertriglyceridemic rats (Šimko *et al.* 2002, Zicha *et al*. 2006b) and rabbits with aortic insufficiency (Šimko *et al.* 1997, 1998).

The thiol group might contribute to the benefits achieved by captopril. Thiols protect NO from oxidation by scavenging oxygen-free radicals and by forming nitrosothiols, both effects prolonging NO half-life and duration of NO action (Zhang and Hogg 2005, Pecháňová 2007, Sládková *et al.* 2007). Interestingly, aldosterone receptor blocker, spironolactone, was also able to prevent degradation of thiol groups and to increase the expression of endothelial NO synthase protein, the effects associated with blood pressure reduction (Pecháňová *et al.* 2006a, Török *et al.* 2007). It seems that not the absolute NO production but the relative balance between vasodilators and vasoconstrictors is decisive.

In our experiments, prevention of both blood pressure increase and cardiovascular remodeling by chronic treatment with antioxidant, provinol, was associated with increased NO synthase activity and enhanced expression of endothelial NO synthase (Pecháňová *et al*. 2004a). It has also been documented that polyphenols of red wines strongly inhibit the synthesis of endothelin-1, a vasoactive peptide that is crucial for the development of coronary atherosclerosis (Corder *et al*. 2001). These data suggest that reduced oxidative stress due to antioxidant action of provinol, its ability to increase endothelial NO synthase activity and to decrease endothelin-1 synthesis may contribute to its antihypertensive effect and protection against cardiovascular remodeling in NO-deficient rats (Pecháňová *et al*. 2006b). Another antioxidant N-acetylcysteine completely prevented L-NAME-induced hypertension, while its therapeutic effect in established L-NAME hypertension was only moderate, although this treatment restored NO synthase activity and lowered conjugated dienes in the heart and kidney (Rauchová *et al*. 2005). Similarly, in SHR chronic administration of N-acetylcysteine partially attenuated the blood pressure increase in young rats, while its effect was negligible in adult SHR with fully developed hypertension. Since chronic N-acetylcysteine and also melatonin treatment had better preventive than therapeutic effects, it seems that ROS play a more important role in the induction than in the maintenance of hypertension. Therefore, the antioxidant treatment is expected be more efficient in the prevention than in the reduction of established hypertension (Pecháňová *et al.* 2006c, 2007).

In accordance with the hypothesis that nitric oxide plays an important role in the central regulation of sympathetic tone, we demonstrated that reduced NO production in the central nervous system of rats with L-NAME-induced hypertension is reflected by enhanced sympathetic vasoconstriction (Pecháňová *et al.* 2004b, 2006b, Kuneš *et al.* 2004, Zicha *et al.* 2006a). The balance between NO and angiotensin II in the vasomotor centers seems to play important role in the regulation of the sympathetic tone.

Conclusions

There is no doubt that endothelium plays a regulatory and protective role by generating vasorelaxing substances. However, under pathophysiological processes and circumstances endothelium-derived vasoconstricting factors can dominate and contribute to deleterious effects. Thus, the balance between vasodilating and vasoconstrictive substances appears to be inevitable for the maintenance of the physiological state of the circulation. Understanding the processes that regulate

balance of vasoactive substances in peripheral vessels and central nervous system may result in more sophisticated approach to the treatment of hypertension and target organ damage.

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