REVIEW

Nitric oxide modulation of metabolic and haemodynamic balance

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Abstract

Nitric oxide (NO) belongs to signal molecules and modulates notably rapid and dynamic processes. Interests are focused on the decreased NO synthesis by endothelial and mitochondrial nitric oxide synthases with the metabolic (i.e. insulin resistance, diabetes, energetical dysbalance) and vascular (i.e. hypertension, atheroslerosis) consequences. A significant source of NO are NO-donors (i.e. organic nitrates). A number of antihypertensive drugs stimulates NO production or inhibits the production of its antagonists (angiotensin II, catecholamines), other drugs (i.e. glucocorticoids) inhibit NO production. These interferences are targets of an intensive research with the aim of NO dysbalance prevention, hypertension and metabolic dysbalances correction. While the clinical research concentrates on NO and insulin resistance, the molecular biological research concentrates on "mitochondrial medicine" with the ambition to formulate a new theory of aging, carcinoma, and other fundamental biological processes *(Fig. 1, Ref. 47)*.

Key words: nitric oxide, nitric oxide synthase, hypertension, insulin resistance, metabolic syndrome.

Endocrine function of endothelial cells was discovered at the end of the previous century. Nitric oxide (NO) was found to be the effective modulator and R.F. Furchgott with L.J. Ignarro (1989) and with F. Murad received a Nobel prize – 1998 for their discoveries. Profesor Ignarro red a lecture at an international meeting also in Bratislava. NO participation in kidney blood flow regulation was presented by our group at that occasion (Dzúrik and Spustová, 2001). However, the search culminates just at the present time (Estévez and Jordán, 2002; Ignarro, 2002). The regulatory significance of NO was discovered also in mitochondria (Ramachandran et al, 2002) and even in plants (Tuteja et al, 2004).

NO synthesis

NO is synthesized from arginine by nitric oxide synthase (NOS). Four NOS have been discovered (Estévez and Jordán, 2002; Ghafourifar and Cadenas, 2005) (Fig. 1): a) *Constitutive Ca-dependent NOS in neuronal cells* (nNOS, NOS I). Its participation in Alzheimer (Yaffe et al, 2004) but notably Parkinson disease is intensively studied. b) *Constitutive Ca-dependent NOS in endothelial cells* (eNOS, NOS III). eNOS synthesizes NO for the needs of cardiovascular system and metabolism. The decreased NO production reflects in hypertension, ischemic heart disease (cardiovascular) and insulin resistance (IR), atherosclerosis (metabolic) alterations (Kvasnička, 2003). eNOS essential cofactor is tetrahydrobiopterine (BH4), obligatory for NO synthesis. However, BH4 participates also in the production of superoxide which inactivates NO (Shinozaki et al, 2004). c) *Constitutive Ca-dependent NOS in mitochondria of various cells* (mtNOS). It is related to nNOS, but surely distinct. Mitochondrial localization, relation to energetical processes, late development of clinically apparent diseases, i.e. diabetes type 2 (DM2), atherosclerosis, other metabolic alterations (Zeviani and Spinazzola, 2003; Duchen, 2004; Ghafourifar and Cadenas, 2005) make it interesting for the search in mentioned diseases. d) *Inducible Ca-independent NOS* (iNOS, NOS II) present in macrophages participates in antimicrobial NO activity.

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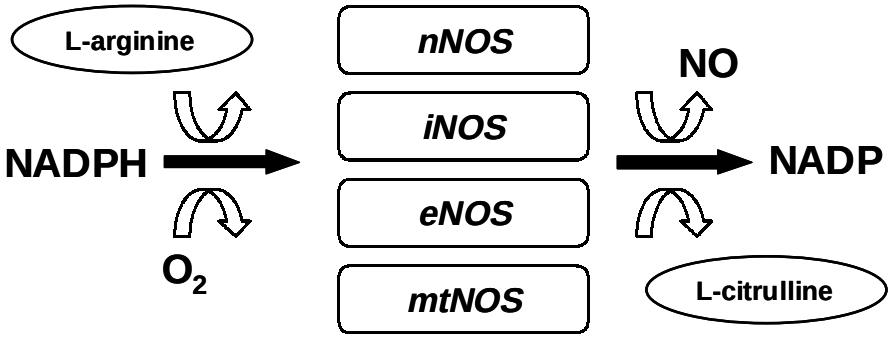

Fig. 1. Nitric oxide synthesis. NO — nitric oxide, nNOS — neuronal nitric oxide synthase, iNOS — inducible nitric oxide synthase, eNOS — endothelial nitric oxide synthase, mtNOS — mitochondrial nitric oxide synthase.

The effects of NO produced by eNOS

NO synthesized in endothelial cells diffuses into the adjacent smooth muscle cells with their functional (blood pressure decrease) and metabolic (decreased IR, dyslipoproteinemia) consequences (Stuhlinger et al, 2002; Wang et al, 2004). An inverse correlation was detected between plasma NO concentration and IR (Cook and Scherrer, 2002; Zavaroni et al, 2004). Their relationship was found even in patients with DM2, (Kashyap et al, 2005). NO is oxidised with the formation of reactive products, the best defined are *peroxynitrite* and *nitrosylated thiols.* Their biological effects were not satisfactorily elucidated yet.

NO mechanism of action has not been elucidated yet in details (Estévez and Jordán, 2002): NO stimulates the acivity of *guanylyl cyclase* with the consequent increased concentration of cyclic guanosine monophosphate (cGMP), and the relaxation of smooth muscle cells (Furchgott and Vanhoutte, 1989).

NO determination is accessible by the Gries reaction after the removal of proteins by various purification procedures (membrane filtration, chromatography, etc). The NO plasma levels are often predicted just on the basis of insulin sensitivity (IS) determination, because of the relationship between NO plasma levels and IS (Cook and Scherrer, 2002; Zavaroni et al, 2004; Dzúrik et al, 2005).

Clinical manifestations of NO dysbalance

NO deficiency caused by the decreased eNOS expression and/or activity is manifested by the energetical dysbalance, IR, hypertension and other functional and metabolic alterations which form a metabolic syndrome (Tkáč, 2005).

The relationship of NO deficiency with IR was already published (Cook and Scherrer, 2002; Zavaroni et al, 2004; Dzúrik et al, 2005) with the glucose and insulin determinations in fasting blood.

Hypertension

NO decreases blood pressure. The administration of arginine antagonist N-monomethyl arginine increases blood pressure up to 40 % because of lack of NO antihypertensive effect (Anggard, 1994). The NO production depends on several factors: a) *Arginine reserves*: Arginine is an amino acid synthesized in urea synthesis cycle. The limiting step appears to be a transmembrane transport of arginine into the endothelial cell (Moss et al, 2004). Arginine supplementation decreases blood pressure (Lekakis et al, 2002). Arginine lack could be caused also by the increased arginine breakdown in arterioles (Johnson et al, 2005). In this relation asymmetric dimethylarginine (ADMA), an endogenous *NOS inhibitor,* is to be mentioned. Its plasma concentration correlates with IR and inverse correlation was found in the decreased kidney function (Zoccali et al, 2001). b) *eNOS synthesis*: Regulation is localized also at the level of eNOS gene. The best known is the eNOS gene polymorphism Glu298Asp. Though the Asp298 shares equivalent enzymatic activity as Glu298, it is more rapidly destroyed than Glu298. Consequently Asp298 carriers suffer from the high risk of cardiovascular or cerebrovascular events (Hingorani, 2003). Moreover patients with DM2 suffer from the decreased eNOS activity (Kalinowski et al, 2003). c) A significant eNOS product is also *superoxide* (Cuzzocrea et al, 2004; Ignarro, 2002) which accelerates NO oxidation, development of hypertension both in human (Brands et al, 2004) and in experimental animals (Cuzzocrea et al, 2004).

High NO hypertension: Though most of hypertensive patients suffer from the low NO plasma concentrations (Štefíková et al, 1993; Zavaroni et al, 2004), there are also exceptions (Bajza et al, 2004): In a controlled study of 71 hypertensive patients compared with 21 IR normotenive patients, the hypertensive IS patients did have similar NO plasma levels and NO urinary excretions. Surprisingly hypertensive IR patients suffered from high plasma levels and urinary NO excretions. These patients suffered from the resistant hypertension. This study presents a discrepance which is to be confirmed. However, there are some experimental results which make this contradictory observation possible. For instance single dose of NO donor increases IS, while continuous 7 day administration potentiated IR (Bajza et al, 2004).

Essential hypertension: Hypertension is one of the signs of metabolic syndrome. However, the evidence on the direct relationship between essential hypertension and NO deficiency is

missing. In the study from our department hypertension was found only in 60 % of IR subjects. On the other hand hypertensive patients do not suffer always from IR (Štefíková et al, 1993). Thus, it appears that hypertension in low NO producers is just an additional secondary hypertension.

Systolic hypertension: The prevalence is highest in old age. Glycerol trinitrate decreases notably systolic blood pressure without a marked decrease of diastolic blood pressure (Stokes, 2004). Unfortunately, the resistance to treatment develops early, like the resistance of angina treated by nitrate donors. The long term effectiveness of slow donors in systolic hypertension has not been evaluated yet, though it appears to be probable.

Nephrogenic hypertension: It is caused by the retention of Na+. Its bad prognosis without effective treatment is beyond doubt. Insufficient NO synthesis in macula densa and the lack of NO in juxtaglomerular cells stimulates vas afferens constriction and participates in hypertension development and resistance to treatment (Dzúrik and Spustová, 2001; Merta et al, 2003; Shimada et al, 2003; Delles et al, 2004).

Antihypertensive drugs

The decreased NO production in hypertension has stimulated the search for the effects of various antihypertensive drugs in NO production.

Glyceroltrinitrate (Stokes, 2004) decreases the systolic blood pressure even if added to antihypertensive therapy. Unfortunately, tolerance to treatment develops soon (Ramachandran et al, 2002). This tolerance is prevented by the asymmetric administration of glyceroltrinitrate or by the prescription "slow NO donors" like isosorbide mononitrate (Stokes, 2004), pentaerythrityl tetranitrate (Kristek et al, 2003) or molsidomine (Gerová and Kristek, 2001; Kristek and Varga, 2001; Chander and Chopra, 2005). However, if NO concentration increases for a long time, IR develops (Cook and Scherrer, 2002). This development has not been elucidated yet and reflects in the prescription of drugs which modulate primarily the NO action and not NO plasma concentration.

ACE inhibitors and AT1-receptor antagonists: They increase NO production not only in nephrogenic but also in essential hypertension.(Slaninka-Miceska et al, 2003; Yavuz et al, 2003). The long term loss of effectiveness is inapparent.

Alfa-blockers: They markedly stimulate IS or inhibit IR (Wangensteen et al, 2002). However, their therapeutic effectiveness is primarily caused by the blockade of epinephrine action on alfa-receptors.

Beta-blockers: The short acting beta-blockers inhibit NO production and decrease IS, while long-lasting beta-blockers and notably hybride beta-blockers carvedilol (Kalinowski et al, 2003) or nebivolol (Fratta Pasini et al, 2005) increase NO production.

Ca antagonists: Dihydropyridines a benzotiazepines increase, while phenylalkylamines do not influence NO production (Ding and Vaziri, 2000).

Inhibitors of NO production: A number of drugs inhibits NO production, i.e. glucocorticoids and immunosuppressive drugs. Systematic search in this area is intensive.

Perspectives of the clinical research

Clinical research usually waits for the results of preclinical studies. However, in this topic preclinical and clinical research are investigated almost simultaneously. The lack of adequate experimental models, the need to control experimental results continuously and the serious consequences of the present clinical procedures require continuous confrontations.

Nitric oxide studies

There is a number of urgent tasks to extend the present knowledge:

a) *NO plasma levels* determination in patients with metabolic syndrome or early isolated disorders, such as IR, hypertension, dyslipoproteinemia.
b) *Nitrite/nitrate urinary excretion*: It is of interest to evaluate especially the renal mechanisms of their excretion and the significance of kidney function impairement.
c) *Acute and chronic effects of model drugs*: This is probably the most urgent task for clinical practice if we realize the need of hypertension prevention and treatment.

The research of individual drug actions proceeds and reaches already the clinical applications.

Insulin resistance studies

The recognition of IR risks pointed to IR decisive role in the development of atherosclerosis and DM2. The mechanism was not apparent and it was elucidated just recently (Wang et al, 2004): a) Insulin modulates glucose metabolism after binding to insulin receptor with a chain of following rections inhibited in IR:

Insulin receptor → Insulin receptor substrate → PI 3-kinase →…

NO deficiency induces IR compensated at least partially by the increased insulin production and increased insulin plasma levels. b) Insulin shares also other action: It stimulates prenylation of protein participating in proliferation. This insulin stimulation is not inhibited in IR! It depends just on plasma insulin concentrations and at IR with increased insulin plasma levels the proliferative atherogenic processes are accelerated.

Thus, *the prevention and therapy of the altered glucose utilization should not be directed to the increase of insulin plasma concentration but to the increase of IS.*

Perspectives of the molecular biological research

The recent study of metabolic syndrome has shawn that both IR and NO dysbalance are caused by mitochondrial genome alteration (Wilson et al, 2004) with the suppressed mitochondrial RNA (mtRNA) synthesis. The study confirmed some independent suggestions (Yavuz et al, 2003; Hampton, 2004) and supports the hypothesis of "mitochondrial medicine" (Gazdíková and Dzúrik, 2004). Hypothesis shares even ambitions to formu-

late a new theory of aging, carcinoma development and other medical and biological problems which do not fit with classical genetic laws (Wilson et al, 2004).

The research of IR and NO production with the following development of metabolic syndrome, DM2 and/or cardiovascular damage shares already its application in clinical medicine, i.e. atherosclerosis and DM2 with clinical consequences, which form the dominant cause of morbidity and mortality in developed countries. Moreover, it opens new molecular biological areas of decisive importance for medicine and even biological sciences.

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