medicinska revija
medical review

UDK: 577.21:546.172.6
ID = 174057740

Mušicki B. et al Med Data Rev 2010;2(1): 29-34 *MEDICAL DATA / Vol. 2. No 1 / Mart 2010.*

Opšti pregledi/
General reviews

ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) REGULATION

REGULACIJA ENDOTELIJALNE AZOT MONOKSID SINTETAZE (eNOS)

Biljana Mušicki, Ph.D., Arthur L. Burnett, M.D.

The Johns Hopkins University, Department of Urology, Baltimore, MD 21287

Correspondence to:
Biljana Musicki, Ph.D., Johns Hopkins Hospital, Department of Urology, Marburg 414, 600 North Wolfe Street, Baltimore, MD 21287, Telephone: 410-955-0352, Fax: 410-614-3695,

E-mail: bmusicki@jhmi.edu

Abstract

Nitric oxide (NO), produced by endothelial NO synthase (eNOS), plays a crucial role in controlling vascular tone and homeostasis. The signaling mechanisms involved in the regulation of eNOS activity and NO production are complex and interdependent. eNOS mRNA levels are controlled both at the transcriptional and posttranscriptional levels. eNOS enzymatic activity is intimately controlled by posttranslational modifications including calcium/calmodulin; phosphorylation; nitrosylation; acylation; interaction with the substrate, cofactors, regulatory proteins; subcellular localization; as well as regulation by reactive oxygen species. Dysregulation of any of these processes leads to reduced bioavailability of endothelial NO in the vasculature, which is a characteristic feature of numerous pathophysiological disorders such as hypertension, diabetes, insulin resistance, obesity, and hyperlipidemia. This review summarizes recent findings relating to molecular mechanisms which regulate eNOS activity.

Key words
posttranslational modification, subcellular targeting, phosphorylation, caveolin-1, heat shock protein 90

ENZYMATIC FUNCTION OF NOS

Endothelial nitric oxide (NO) synthase (eNOS) is a member of a family of three mammalian nitric oxide synthase (NOS) isoforms that catalyze the production of NO from L-arginine using molecular oxygen. The three NOS isoforms are neuronal NOS (nNOS or NOS I), expressed primarily in brain and skeletal muscle, inducible NOS (iNOS or NOS II), expressed in macrophages, liver, and vascular smooth muscle, and endothelial NOS (eNOS or NOS III), expressed in endothelial cells and cardiac myocytes. The constitutive forms of the enzyme, nNOS and eNOS, are coupled to calcium and calmodulin, while iNOS is independent of calcium and calmodulin and requires new protein synthesis [(1)]. The three isoforms are the products of distinct genes. Although these isoforms share approximately 50–60% sequence identity and the same basic structural mechanisms, they contain distinctive catalytic and regulatory properties which characterize them.

In common with all 3 NOS enzymes, eNOS is a homodimeric oxidoreductase. Each monomer has two functionally different domains, an N-terminal oxygenase and a C-terminal reductase domain. The N-terminal domain binds heme, a cofactor 5,6,7,8-tetrahydrobiopterin (BH4), heme, molecular oxygen, and the substrate L-arginine. L-arginine binds at the enzyme active site near heme, while molecular oxygen is directed to the ferrous heme iron. The binding sites for BH4 and heme are localized along the interface of the monooxygenase domains of the dimeric, active form of NOS. The C-terminal reductase domain binds nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). The N- and C-terminal domains are linked by a short sequence that binds calmodulin, an allosteric effector that is essential for full NOS activity. eNOS catalyzes electron transfer from the C-terminally bound NADPH via the flavins to the heme on the N terminus of the other monomer. Calcium/calmodulin activation causes conformational changes in the enzyme and increases the rate of electron flow. At the heme, electrons reduce and activate oxygen, resulting in oxidation of L-arginine to NO and L-citrulline [(2)]. Because electrons flow from the reductase domain of one NOS monomer to the oxygenase domain of another NOS monomer, enzyme dimerization is required for full enzymatic activity.

The discovery of "nitric oxide as a signalling molecule in the cardiovascular system" resulted in the Nobel Prize in Physiology or Medicine for 1998 awarded to Robert

Furchgott, Louis J. Ignarro and Ferid Murad. Endothelial NO regulates vasodilation and maintains basal vascular tone, and inhibits leukocyte adhesion to the vascular endothelium, platelet aggregation, smooth muscle cell proliferation and migration, and endothelial cell apoptosis. In the vascular system, NO-dependent relaxation of vascular smooth muscle is predominantly soluble guanylyl cyclase and protein kinase G dependent. Since its identification and cloning exhaustive efforts have been put forth to understand the processes mediating eNOS function in vascular endothelium, in certain types of epithelium, and in other cells such as cardiac myocytes.

eNOS Regulatory Mechanisms

The human eNOS gene contains 26 exons that span 21 kb [3]. eNOS mRNA is 4052 nucleotide long and has a half-life of 10-35 hours. Although initially considered to be a constitutive enzyme, eNOS is regulated by a variety of exogenous stimuli.

eNOS is controlled through its transcriptional and post-transcriptional regulation, and posttranslationally through the regulation of its activity. In cultured endothelial cells cytokines, lipopolysaccharide, and oxidized low density lipoprotein (LDL) downregulate eNOS expression. In contrast, shear stress, estrogen, and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors upregulate eNOS expression. Cytokines, lipopolysaccharide, tumor necrosis factor alpha, and oxidized LDL decrease eNOS expression at the posttranscriptional level by reducing the stability of eNOS mRNAs [4].

Protein expression of eNOS, however, may not necessarily reflect the functional state of the enzyme, and endothelial NO synthesis may be regulated independently of changes in eNOS enzyme activity. Changes in endothelial NO production may result from specific posttranslational modifications of endogenously expressed eNOS. In addition to rapid regulation by calcium/calmodulin, posttranslational mechanisms include substrate and cofactor availability, fatty acid modification (such as myristoylation and palmitoylation), subcellular localization, dimerization of the enzyme subunits, binding to other proteins (such as caveolin-1 and heat shock protein 90 [HSP90]), and phosphorylation, as well as regulation by reactive oxygen species (ROS; 5). These mechanisms regulate eNOS-mediated responses under physiologic circumstances and provide various mechanisms whereby endothelial NO availability may be altered in states of vasculogenic dysfunction. Table 1 summarizes the major molecular mechanisms of eNOS posttranslational regulation which regulate eNOS activity and NO bioavailability. Although listed as separate categories, these regulatory mechanisms are not mutually exclusive; rather they may be interrelated to maintain the proper spatial and temporal organization of eNOS signaling.

The following review describes the major mechanisms of eNOS posttranslational modification and their effect on eNOS activity in the vasculature.

1) Calcium-dependent eNOS activation

Activation of eNOS with calcium/calmodulin is essential to the first step of the catalytic process of NO production. The activation of eNOS in response to agonists such as acetylcholine, bradykinin, and sphingosine 1-phosphate, is induced by increases in intracellular calcium resulting from activation of G-protein-coupled receptors or from mobilization from intracellular calcium stores. Calcium forms a complex with calmodulin, which subsequently binds to the calmodulin-binding site on eNOS. Calmodulin binding to eNOS facilitates electron transfer from the reductase to the oxygenase domain, and simultaneously disrupts the inhibitory eNOS/caveolin-1 interaction [6]. As intracellular calcium concentrations rapidly decline, calmodulin dissociates from eNOS, resulting in a rapid and transient production of endothelial NO.

eNOS posttranslational regulation

- **Rapid calcium/calmodulin regulation**
- **Substrate and cofactor availability**
- **Phosphorylation**
- **Intracellular localization**
- **Protein-protein interaction**
  - **Negative regulators: Caveolin-1, NOSIP, NOSTRIN, CHIP**
  - **Positive regulators: HSP90, Dynamin, Porin**
- **Fatty acid modification**
  - **N-Myristoylation**
  - **Cysteine palmitoylation**
- **Regulation by ROS**
- **Regulation by asymmetric dimethylarginine (ADMA)**
- ***O*-linked glycosylation**
- **S-nitrosylation**

Table 1. Major molecular mechanisms of eNOS posttranslational regulation

2) eNOS phosphorylation

Phosphorylation of eNOS at multiple sites is a critical mechanism regulating eNOS activity. The phosphorylation is regulated by diverse signaling pathways where specific protein kinases and protein phosphatases regulate phosphorylation at each site. A diverse and growing list of activators has been identified that regulate eNOS phosphorylation and NO synthesis (Figure 1).

Seven specific sites of phosphorylation have been identified on eNOS: Tyr-81, Ser-114, Thr-495, Ser-615, Ser-633, Tyr-657, and Ser-1177 (human sequence, equivalent to Tyr-83, Ser-116, Thr-497, Ser-617, Ser-635, Tyr-659, and Ser-1179 on bovine eNOS). Phosphorylation of these sites increases or decreases the enzyme's activity directly or by modulating other regulatory sites on eNOS. eNOS stimulatory phosphorylation sites include Ser-1177, Ser-633, Ser-615, and Tyr-81; eNOS inhibitory phosphorylation sites include Thr-495, Ser-114, and Tyr-657.

The best characterized eNOS phosphorylation site is Ser-1177, located in the reductase domain of the enzyme. In unstimulated endothelial cells, Ser-1177 is not phosphorylated. The most important physiologic stimulator of eNOS phosphorylation at this residue is shear stress, a pressure exerted on endothelial cells by blood flow in the vessel at a constant flow rate. Shear stress, as well as several hormones and growth factors, such as vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), statins, and estrogens, phosphorylate eNOS on this site. Ser-1177 phosphorylation results in increased electron flux through the reductase domain of the enzyme and reduced calmodulin

dissociation from eNOS. As a result, eNOS becomes more active and produces more NO, even at resting levels of intracellular calcium [7, 8]. Ser-1177 can be phosphorylated by numerous protein kinases, including Akt (protein kinase B), cyclic AMP-dependent protein kinase (PKA), AMP-activated protein kinase (AMPK), cGMP-dependent protein kinases (PKG), calcium/calmodulin-dependent protein kinase II (CaMKII), and protein kinase C [9]. The relative contributions of these different kinase pathways are not completely understood, but it appears that different extracellular stimuli activate distinct kinases. For example, while shear stress phosphorylates eNOS at Ser-1177 mostly by PKA, insulin, estrogen, sphingosine 1-phosphate, and VEGF mainly phosphorylate this same residue by Akt. The bradykinin-, calcium ionophore-, and thapsigargin-induced phosphorylation of Ser-1177, on the other hand, are mediated by CaMKII [10].

The physiological significance of phosphorylation of other sites on eNOS is less known. Ser-633 is located within one of the auto-inhibitory loops on eNOS, which is folded in such a way as to physically impede the access of calmodulin to its binding domain. Phosphorylation of this residue by PKA increases eNOS activity [11, 12]. Various agonists, such as shear stress, VEGF, bradykinin, and statins, increase phosphorylation of this site after initial activation of the enzyme by calcium flux and/or Ser-1177 phosphorylation.

Phosphorylation of eNOS at Ser-615 by PKA and Akt increases eNOS activity by increasing the calcium/calmodulin sensitivity of the enzyme, and appears to be important for regulating interactions between eNOS and other proteins, and for phosphorylation of eNOS at other sites [11, 12].

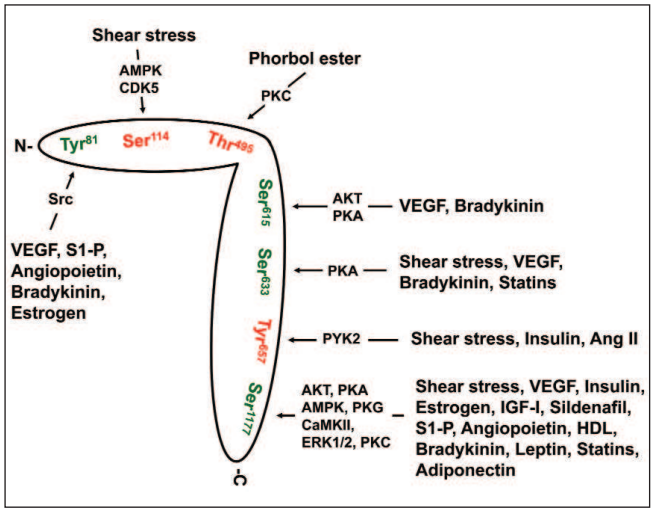

Figure 1. Regulation of eNOS phosphorylation. eNOS stimulatory phosphorylation sites include Ser-1177, Ser-633, Ser-615, and Tyr-81, shown in green. eNOS inhibitory phosphorylation sites include Thr-495, Ser-114, and Tyr-657, shown in red. The figure depicts known stimulators of eNOS phosphorylation at specific sites, and protein kinases which mediate phosphorylation at these sites.

VEGF = vascular endothelial growth factor; S1-P = sphingosine 1-phosphate; Ang II = angiotensin II; IGF-I = insulin-like growth factor I; HDL = high density lipoprotein; AMPK = AMP-activated protein kinase; CDK5 = cyclin-dependent kinase 5; PKC = protein kinase C; PKA = protein kinase A; PYK2 = proline-rich tyrosine kinase 2; PKG = protein kinase G; CaMKII = calcium/calmodulin-dependent protein kinase II; ERK1/2 = extracellular signal-regulated kinase

eNOS phosphorylation at Tyr-81 contributes to an increase in eNOS activity. This effect does not seem to be direct, but rather Tyr-81 phosphorylation on eNOS modulates the sensitivity of the enzyme to calcium, alters eNOS-protein interactions, or changes the subcellular localization of the enzyme [13]. Src-dependent phosphorylation of eNOS at this residue occurs in response to a number of agonists, including thapsigargin, VEGF, bradykinin, ATP, sphingosine 1-phosphate, estrogen, angiopoietin, and acetylcholine.

Thr-495 residue, which is within the calmodulin binding sequence of eNOS, is constitutively phosphorylated in endothelial cells. eNOS phosphorylation at Thr-495 reduces eNOS catalytic activity by interfering with binding of calcium/calmodulin to eNOS [14-16]. Reciprocal dephosphorylation of Thr-495 and phosphorylation of Ser-1177 appears to be essential for eNOS activity. PKC signaling has commonly been accepted to promote eNOS phosphorylation at Thr-495 and dephosphorylation at Ser-1177, reducing the enzyme's activity [15]. Agonist-induced dephosphorylation of Thr-495, associated with increased eNOS activity, is mediated by Ser/Thr protein phosphatase 1 (PP1), Ser/Thr protein phosphatase 2A (PP2A), and calcineurin (PP2B; 15). Recently, the dephosphorylation of Thr-495 has been linked to eNOS uncoupling (i.e., superoxide production by eNOS), however, it remains to be determined whether this occurs in vivo [17].

Phosphorylation of eNOS at Ser-114 inhibits agonist-induced, but not basal, enzyme activity [18]. This inhibition does not occur through a direct effect on catalytic activity of the enzyme, but rather indirectly through an increased association of eNOS with caveolin-1. Deposphorylation of eNOS on Ser-114 by the phosphatase calcineurin activates eNOS.

Tyr-657 is located within the FMN-binding domain of eNOS. Phosphorylation of eNOS on Tyr-657 by activation of proline-rich tyrosine kinase 2 (PYK2) attenuates the activity of the enzyme. A number of physiologically relevant stimuli lead to the activation of PYK2 in endothelial cells, including shear stress, insulin, angiotensin II, and oxidative stress [19]. Given that shear stress and insulin elicit the phosphorylation of both Ser-1177 and Tyr-657 residues, which positively and negatively regulate eNOS activity, respectively, it is tempting to speculate that phosphorylation of eNOS on Tyr-657 in response to stimuli such as shear stress and insulin plays a key role in negatively modulating enzyme activity, thus keeping NO output low and reducing the risk of depletion of eNOS substrate and essential cofactors.

3) eNOS Trafficking and Protein-Protein Interaction

Caveolae and Caveolin-1

The proper subcellular localization of eNOS is critical for optimal coupling of extracellular stimulation of eNOS with NO production. eNOS is unique among the NOS isoforms in that it is the only NOS enzyme that is modified by cotranslational myristoylation and posttranslational palmitoylation. These modifications are responsible for targeting eNOS to the plasma membrane and to intracellular membranes, including the Golgi. In endothelial cells and in isolated blood vessels, the majority of eNOS is present in these

two locations [20]. eNOS localized to the plasma membrane is constitutively phosphorylated at Ser-1177 and produces significantly more NO compared to the Golgi pool of eNOS, while its activity is significantly reduced in areas where eNOS is normally absent, including the cytosol, mitochondria, and nucleus.

In plasma membranes, the majority of functional eNOS is targeted to cholesterol-rich microdomains termed caveolae [21]. Caveolin-1, the resident membrane protein of caveolae, can directly interact with eNOS and inhibit its activity [22]. These in vitro findings are supported by recent genetic data showing that caveolin-1 knockout mice exhibit increased basal and stimulated eNOS activation and endothelium-dependent relaxations [23], while mice overexpressing caveolin-1 exhibit reduced eNOS activation and NO-dependent vascular function [24]. Receptor activation, or humoral and mechanical stimuli such as estrogen and shear stress, induce redistribution of eNOS from plasma membrane caveolae (probably assisted by dynamin) and
away from its tonic inhibition to intracellular membranes. eNOS translocation is associated with interaction with other proteins, such as calmodulin and HSP90, and dephosphorylation of Thr-495, allowing calcium/calmodulin binding to eNOS, phosphorylation of positive regulatory sites (such as Ser-1177), denitrosylation, and eNOS activation. After activation most eNOS returns to the membrane fraction [25].

Whereas caveolin-1 interaction with eNOS is required to prevent inadequate NO production under basal conditions, it also facilitates the integration of extracellular stimuli to intracellular NO signals. Compartmentalization of eNOS in caveolae is necessary to localize regulatory and signaling proteins, including caveolin-1, certain G-protein coupled receptors, HSP90, Akt, and lipoprotein receptors such as scavenger receptor class B type I (SR-BI) and CD36, and provide their interaction with eNOS [26]. Caveolin-1 is believed to be instrumental in the compartmentalization of signaling molecules within caveolae, thus enabling rapid and selective regulation of calcium- and phosphorylation-dependent signal transduction events that modify the response of the enzyme to extracellular stimuli [27]. Caveolin-1 also binds to cholesterol and is involved in trafficking cholesterol from the endoplasmic reticulum to the plasma membrane to regulate the cholesterol content of caveolae, which in turn determines the structure and function of caveolae [26]. Disruption of the lipid environment within caveolae impairs the activity of eNOS and bioavailability of endothelial NO during conditions of hypercholesterolemia-induced vascular disease and atherosclerosis.

In addition to caveolin-1 which serves as a major negative allosteric regulator of eNOS, the NOS-interacting protein (NOSIP) and the NOS traffic inducer (NOSTRIN) can also negatively regulate eNOS localization in the plasma membrane. The C-terminal HSP70-interacting protein (CHIP) interacts with HSP90 and HSP70 and negatively regulates eNOS trafficking into the Golgi complex [9].

Heat shock protein 90 (HSP90)

Hsp90 acts as a major protein activator of eNOS. HSP90 is associated with eNOS in the resting state, and, upon stimulation of endothelial cells with VEGF, estrogen, histamine, and shear stress, the association between the two proteins is increased, concomitant with enhanced NO production [28]. Blockade of HSP90-mediated signaling inhibits agonist-induced NO production and vasorelaxation in vitro and in vivo in fetal lambs with persistent pulmonary hypertension, while overexpression of HSP90 exerts cardioprotection in a pig model of cardiac infarct [29]. The mechanism of this activation involves calmodulin-dependent disruption of eNOS binding with caveolin-1, recruitment of eNOS and Akt to adjacent regions on HSP90, reduced dephosphorylation of Akt, and increased ability of Akt to phosphorylate HSP90-bound eNOS, which facilitates eNOS activation by Ser-1177 phosphorylation [6, 30]. The direct protein interaction between eNOS and HSP90 also enhances eNOS coupled activity and prevents superoxide production by eNOS [31].

Other proteins associated with increased eNOS activity or NO release are the intracellular trafficking protein dynamin and the voltage-dependent anion/cation channel porin [9].

4) An endogenous inhibitor of eNOS: Asymmetric Dimethylarginine (ADMA)

ADMA is an endogenous inhibitor for NO synthesis. ADMA has been shown to compete with L-arginine, the NOS substrate [32], and recently it has been shown to induce eNOS uncoupling [33]. It is a naturally occurring amino acid resulting from proteolysis of methylated arginine in proteins [32]. The amount of ADMA generated within a cell is dependent on the extent of arginine methylation in proteins and the rates of protein turnover. The methylation of arginine is catalyzed by arginine methyltransferase (PRMT) type I. ADMA is eliminated largely through its metabolism to citrulline by dimethylarginine dimethylaminohydrolase (DDAH)-1 and -2, type 2 being predominant in endothelial cells. Both synthesis and degradation of ADMA are regulated in an active manner; thus dysregulation of either of these pathways may result in elevated levels of ADMA. Increased ADMA levels have been implicated in the pathogenesis of a variety of cardiovascular diseases [34].

5) S-Nitrosylation

S-nitrosylation is the covalent adduction of NO-derived nitrosyl groups to the cysteine thiols of proteins. Protein S-nitrosylation / denitrosylation is now recognized as a regulatory component of signal transduction comparable with phosphorylation / dephosphorylation. S-nitrosylation of eNOS leads to enzyme inhibition, whereas denitrosylation is associated with an increase in enzyme activity. In resting endothelial cells eNOS is tonically inhibited by S-nitrosylation at Cys-94 and Cys-99 (Cys-96 and Cys-101 in bovine eNOS) of the zinc tetrathiolate cluster, which comprises the eNOS dimer interface (35). While the mechanism for S-nitrosylation of eNOS is not well understood, it is possible that it modifies substrate or cofactor binding to eNOS, or decreases the efficiency of electron transfer at the interface between the eNOS monomers (1, 35). Agonists such as VEGF and insulin rapidly and transiently decrease eNOS S-nitrosylation concomitant with the increase in eNOS phosphorylation at Ser-1177 and enzyme activation. Although eNOS denitrosylation occurs simultaneously with Ser-1177 phosphorylation, these modifications do not appear to be interdependent [35]. eNOS is subsequently renitrosylated,

corresponding to the decline in eNOS Ser-1177 phosphorylation and the return of the enzyme's activity to its basal state. The source of NO for nitrosylation is eNOS itself localized in the membranes, implying that this modification may be one of the final steps in returning the enzyme to its basal state after its activation and translocation.

6) eNOS uncoupling

eNOS (as well as nNOS and iNOS) can switch from a NO- to a superoxide-producing enzyme, a phenomenon termed eNOS uncoupling. In the uncoupled state, electrons are diverted to molecular oxygen rather than to L-arginine, resulting in the preferential superoxide production over NO production. eNOS uncoupling, thus, refers to both the impaired NO-producing activity of the enzyme as well as its increased capacity to produce superoxide [36]. This decreases the abundance of the active dimeric form of eNOS, while total eNOS expression is retained, or even increased, but mainly in the monomeric form. Decreased NO production and increased superoxide production by uncoupled eNOS further enforce oxidative stress by perpetuating eNOS uncoupling through peroxynitrite formation, thereby resulting in chronic endothelial dysfunction [37].

The mechanism underlying eNOS uncoupling is the subject of intense investigation. Oxidation and nitrosylation of the zinc thiolate cluster of eNOS can release zinc and presumably form disulfide bonds between the monomers, destabilizing the eNOS dimer. The phosphorylation status of eNOS at Thr-495 may also determine whether eNOS produces NO (phospho 495) or superoxide (dephospho 495). Increased association of HSP90 with eNOS has been shown to "recouple" eNOS activity in vitro and in animal studies [36]. Decreased availability of L-arginine has also been suggested to lead to eNOS uncoupling under pathophysiological conditions such as hypercholesterolemia and hypertension, and L-arginine supplementation was found to restore endothelial function [2]. These nonsubstrate effects of L-arginine have been attributed to its direct radical scavenging properties, the cooperativity between L-arginine and BH4 binding sites on eNOS, and the competition of L-arginine with the endogenous inhibitor of eNOS, ADMA [2]. The best characterized cause of eNOS uncoupling involves the imbalance between BH4 levels and eNOS protein levels. BH4-deficient eNOS, when activated, still receives electrons from NADPH and donates them one at a time to molecular oxygen instead of L-arginine, resulting in one-electron reduction to form superoxide anion. The mechanisms leading to reduced BH4 availability in conditions associated with endothelial dysfunction are incompletely understood, but may relate to decreased BH4 biosynthesis or enhanced oxidative degradation of BH4 to 7,8-dihydrobiopterin [38, 39]. eNOS uncoupling accompanied by depletion of BH4 is currently thought to play an important role in vascular disorders including diabetes, hypercholesterolemia, atherosclerosis, hypertension, and aging. Accordingly, BH4 supplementation restores eNOS-dependent blood flow in aging people, in patients with diabetes, hypercholesterolemia, and hypertension, in chronic smokers, and in animal models of vascular disorders [39, 40].

SUMMARY

Constitutively expressed eNOS produces low concentrations of NO, which plays a critical role in vascular function and vascular homeostasis. Multiple mechanisms dynamically regulate the enzymatic function of eNOS in spatial and temporal manners to tightly control NO bioavailability in the vessel wall in response to extracellular stimuli. eNOS activity is dependent on calcium/calmodulin activation, availability of its substrate L-arginine and cofactors (NADPH, FAD, FMN, and BH4), protein-protein interactions, fatty acylation (myristoylation and palmitoylation), S-nitrosylation, phosphorylation, and regulation by ROS. These multiple molecular mechanisms act in concert to affect eNOS activity and prevent potentially deleterious NO overproduction or underproduction. Endothelial dysfunction, which refers mostly to diminished endothelial NO bioavailability in the vessel wall, is an early sign of vascular diseases such as hypertension, hyperlipidemia, and diabetes. Thus, the endothelium is a novel therapeutic target for the treatment of cardiovascular diseases associated with endothelial dysfunction. Further elucidation of the molecular mechanisms of eNOS regulation is crucial for better understanding of NO-mediated responses in vascular tissues under physiologic and pathophysiologic circumstances.

Apstrakt

Azotni monoksid (NO), koji proizvodi enzim endotelijalna azot monoksid sintetaza (eNOS), ima kriticnu ulogu u kontroli vaskularne funkcije i homeostaze. Signalni mehanizmi koji kontrolisu aktivnost eNOS i produkciju NO su kompleksni i medjuzavisni. Nivo eNOS mRNK je regulisan na transkripcionom i na posttranskripcionom nivou. Enzimatska aktivnost eNOS podleze posttranslacionoj regulaciji, koja ukljucuje kalcijum/kalmodulin, fosforilaciju, nitrozilaciju, acilaciju, interakciju sa supstratom, kofaktorima, regulatornim proteinima, i slobodnim radikalima, i celijsku lokalizaciju . Poremecena regulacija bilo kog od ovih procesa rezultira u smanjenoj kolicini NO u vaskulaturi, karakteristicna za mnoga patofizioloska stanja kao so su povisen krvni pritisak, dijabetes, neosetljivost na insulin, gojaznost, i povisen holesterol. Ovaj revijski rad sumira molekularne mehanizme koji regulisu aktivnost eNOS.

REFERENCES

1. Dudzinski DM, Igarashi J, Greif D, Michel T: The regulation and pharmacology of endothelial nitric oxide synthase. Annu Rev Pharmacol Toxicol. 2006; 46: 235-276.

2. Fostermann U: Janus-faced role of endothelial NO synthase in vascular disease: uncoupling of oxygen reduction from NO synthesis and its pharmacological reversal. Biol Chem. 2006; 387, 12: 1521-1533.

3. Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, Tsui LC, Schappert KT: Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem. 1993; 268, 23: 17478-17488.

4. Searles CD: Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression. Am J Physiol Cell Physiol. 2006; 291, 5: C803–C816.

5. Michel T, Vanhoutte PM: Cellular signaling and NO production. Pflugers Arch. 2010 Jan 16. ŠEpub ahead of printĆ.

6. Busse R, Mulsch A: Calcium-dependent nitric oxide synthesis in endothelial cytosol is mediated by calmodulin. FEBS Lett. 1990; 265, 1: 133-136.

7. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM: Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature. 1999; 399, 6736: 601-605.

8. Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC: Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. Nature. 1999; 399, 6736: 597-601.

9. Sessa WC: eNOS at a glance. J Cell Sci. 2004; 117, Pt 12: 2427-2429.

10. Fleming I: Molecular mechanisms underlying the activation of eNOS. Pflugers Arch. 2009 Dec 13. [Epub ahead of print].

11. Bauer PM, Fulton D, Boo YC, Sorescu GP, Kemp BE, Jo H, Sessa WC: Compensatory phosphorylation and protein-protein interactions revealed by loss of function and gain of function mutants of multiple serine phosphorylation sites in endothelial nitric-oxide synthase. J Biol Chem. 2003; 278, 17: 14841-14849.

12. Michell BJ, Harris MB, Chen ZP, Ju H, Venema VJ, Blackstone MA, Huang W, Venema RC, Kemp BE: Identification of regulatory sites of phosphorylation of the bovine endothelial nitric-oxide synthase at serine 617 and serine 635. J Biol Chem. 2002: 277, 44: 42344–42351.

13. Fulton D, Ruan L, Sood SG, Li C, Zhang Q, Venema RC: Agonist-stimulated endothelial nitric oxide synthase activation and vascular relaxation: role of eNOS phosphorylation at Tyr83. Circ Res. 2008; 102, 4: 497–504.

14. Harris MB, Ju H, Venema VJ, Liang H, Zou R, Michell BJ, Chen ZP, Kemp BE, Venema RC: Reciprocal phosphorylation and regulation of endothelial nitric oxide-synthase in response to bradykinin stimulation. J Biol Chem, 2001; 276, 19: 16587-16591.

15. Michell BJ, Chen ZP, Tiganis T, Stapleton D, Katsis F, Power DA, Sim AT, Kemp BE: Coordinated control of endothelial nitric-oxide synthase phosphorylation by protein kinase C and cAMP-dependent protein kinase. J Biol Chem. 2001; 276, 21: 17625-17628.

16. Fleming I, Fisslthaler B, Dimmeler S, Kemp BE, Busse R: Phosphorylation of Thr (495) regulates Ca(2+)/calmodulin-dependent endothelial nitric oxide synthase activity. Circ Res. 2001; 88, 11: E68-E75.

17. Lin MI, Fulton D, Babbitt R, Fleming I, Busse R, Pritchard KA Jr, Sessa WC: Phosphorylation of threonine 497 in endothelial nitric-oxide synthase coordinates the coupling of L-arginine metabolism to efficient nitric oxide production. J Biol Chem. 2003; 278, 45: 44719–44726.

18. Li C, Ruan L, Sood SG, Papapetropoulos A, Fulton D, Venema RC: Role of eNOS phosphorylation at Ser-116 in regulation of eNOS activity in endothelial cells. Vascul Pharmacol. 2007; 47, 5-6: 257-264.

19. Fisslthaler B, Loot AE, Mohamed A, Busse R, Fleming I: Inhibition of endothelial nitric oxide synthase activity by proline-rich tyrosine kinase 2 in response to fluid shear stress and insulin. Circ Res. 2008; 102, 12:1520-1528.

20. Fulton D, Fontana J, Sowa G, Gratton JP, Lin M, Li KX, Michell B, Kemp BE, Rodman D, Sessa WC: Localization of endothelial nitric-oxide synthase phosphorylated on serine 1179 and nitric oxide in Golgi and plasma membrane defines the existence of two pools of active enzyme. J Biol Chem. 2002; 277, 6: 4277–4284.

21. Feron O, Belhassen L, Kobzik L, Smith TW, Kelly RA, Michel T: Endothelial nitric oxide synthase targeting to caveolae. Specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells. J Biol Chem. 1996; 271, 37: 22810-22814.

22. Garcia-Cardena G, Martasek P, Masters BS, Skidd PM, Couet J, Li S, Lisanti MP, Sessa WC: Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the nos caveolin binding domain in vivo. J Biol Chem. 1997; 272, 41: 25437-40.

23. Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, Menne J, Lindschau C, Mende F, Luft FC, Schedl A, Haller H, Kurzchalia TV: Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. Science. 2001; 293, 5539: 2449-2452.

24. Bauer PM, Yu J, Chen Y, Hickey R, Bernatchez PN, Looft-Wilson R, Huang Y, Giordano F, Stan RV, Sessa WC: Endothelial-specific expression of caveolin-1 impairs microvascular permeability and angiogenesis. Proc Natl Acad Sci USA. 2005; 102, 1: 204-209.

25. Govers R, Oess S: To NO or not to NO: 'where?' is the question. Histol Histopathol. 2004; 19, 2: 585-605.

26. Everson WV, Smart EJ: Influence of caveolin, cholesterol, and lipoproteins on nitric oxide synthase: implications for vascular disease. Trends Vasc Med. 2001; 11, 6: 246–250.

27. Shaul PW: Regulation of endothelial nitric oxide synthase: location, location, location. Annu Rev Physiol. 2002; 64: 749-774.

28. Garcia-Cardena G, Fan R, Shah V, Sorrentino R, Cirino G, Papapetropoulos A, Sessa WC: Dynamic activation of endothelial nitric oxide synthase by Hsp90. Nature. 1998; 392, 6678: 821-824.

29. Chatterjee A, Black SM, Catravas JD: Endothelial nitric oxide (NO) and its pathophysiologic regulation. Vascul Pharmacol. 2008; 49, 46: 134-140.

30. Balligand JL: Heat shock protein 90 in endothelial nitric oxide synthase signaling. Following the lead(er)? Circ Res. 2002; 90, 8: 838-841.

31. Pritchard KA Jr, Ackerman AW, Gross ER, Stepp DW, Shi Y Fontana JT, Baker JE, Sessa WC: Heat shock protein 90 mediates the balance of nitric oxide and superoxide anion from endothelial nitric-oxide synthase. J Biol Chem. 2001; 276, 21: 17621-17624.

32. Vallance P, Leiper J: Cardiovascular biology of the asymmetric dimethylarginine: dimethylarginine dimethylaminohydrolase pathway. Arterioscler Thromb Vasc Biol. 2004; 24, 6: 1023-1030.

33. Sud N, Wells SM, Sharma S, Wiseman DA, Wilham J, Black SM: Asymmetric dimethylarginine inhibits HSP90 activity in pulmonary arterial endothelial cells: role of mitochondrial dysfunction. Am J Physiol Cell Physiol. 2008; 294, 6: C1407-1418.

34. Leiper J, Nandi M, Torondel B, Murray-Rust J, Malaki M, O'Hara B, Rossiter S,Anthony S, Madhani M, Selwood D, Smith C, Wojciak-Stothard B, Rudiger A, Stidwill R, McDonald NQ, Vallance P: Disruption of methylarginine metabolism impairs vascular homeostasis. Nat Med. 2007;13, 2: 198-203.

35. Erwin PA, Mitchell DA, Sartoretto J, Marletta MA, Michel T: Subcellular targeting and differential S-nitrosylation of endothelial nitric-oxide synthase. J Biol Chem. 2006; 281, 1: 151-157.

36. Schulz E, Jansen T, Wenzel P, Daiber A, Munzel T: Nitric oxide, tetrahydrobiopterin, oxidative stress, and endothelial dysfunction in hypertension. Antioxid Redox Signal. 2008; 10, 6: 1115-1126.

37. Munzel T, Daiber A, Ullrich V, Mulsch A: Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expression of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. Arterioscler Thromb Vasc Biol. 2005; 25, 8: 1551–1557.

38. Schmidt TS, Alp NJ: Mechanisms for the role of tetrahydrobiopterin in endothelial function and vascular disease. Clin Sci (Lond). 2007; 113, 2: 47-63.

39. Katusic ZS, d'Uscio LV, Nath KA: Vascular protection by tetrahydrobiopterin: progress and therapeutic prospects. Trends Pharmacol Sci. 2009; 30, 1: 48-54.

40. Alp NJ, Channon KM: Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. Arterioscler Thromb Vasc Biol. 2004; 24, 3: 413–420.

The paper was accepted 25.02.2010.