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ENDOTHELIAL NITRIC OXIDE (NO) AND ITS PATHOPHYSIOLOGIC REGULATION

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Abstract

Nitric oxide (NO) is a gaseous lipophilic free radical generated by three distinct isoforms of nitric oxide synthases (NOS), type 1 or neuronal (nNOS), type 2 or inducible (iNOS) and type 3 or endothelial NOS (eNOS). Expression of eNOS is altered in many types of cardiovascular disease, such as atherosclerosis, diabetes and hypertension. The ubiquitous chaperone heat shock protein 90 (hsp90) associates with NOS and is important for its proper folding and function. Current studies point toward a therapeutic potential by modulating hsp90-NOS association in various vascular diseases. Here we review the transcriptional regulation of endothelial NOS and factors affecting eNOS activity and function, as well as the important vascular pathologies associated with altered NOS function, focusing on the regulatory role of hsp90 and other factors in NO-associated pathogenesis of these diseases.

Keywords

nitric oxide; hsp90; cardiovascular disease

Transcriptional and post-transcriptional regulation of eNOS in endothelial cells

Endothelial cells have a constitutive expression of eNOS and like other constitutively expressed proteins, the eNOS promoter lacks the typical TATA box. Instead, it has other multiple *cis*-regulatory DNA sequences like SP-1, GATA, activator protein-1, activator protein-2, nuclear factor-1, sheer stress response elements and sterol-regulatory elements (Marsden et al., 1993). The presence of these consensus sites is consistent with evidence showing that levels of eNOS transcripts are elevated by sheer stress (Davis et al., 2001; Woodman et al., 2005), exercise (Sessa et al., 1994; Yang et al., 2002) and hypoxia (Le Cras et al., 1996). Regulation of eNOS transcription by estrogens is still a matter of debate (Arnal et al., 1996; Kleinert et al., 1998), however, estradiol relaxes rat aortic segments via endothelium-dependent and -independent mechanisms involving the NO-cGMP signaling system (Abou-Mohamed et al., 2003). Both lipopolysaccharide (Arriero et al., 2000) and tumor necrosis factor- α (Yoshizumi et al., 1993) decrease eNOS gene expression by reducing the stability of eNOS mRNAs. Basal human eNOS transcription is controlled by two regulatory regions, the positive regulatory

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domains I and II (PRD I, PRD II). These regulatory domains bind various types of transcription and *trans*-acting factors and regulate eNOS transcription by complex *cis* and *trans* interactions (Searles, 2006). Moreover, these regions also contain differentially methylated nucleotides that restrict eNOS transcription largely in vascular endothelial cells (Chan et al., 2004).

The constitutively expressed eNOS mRNA is about 4052 nucleotide long and has a half-life of 10-35 hours. Therefore, synthesis of the encoded proteins is likely to persist long after gene expression has been repressed. Thus, altering the half-life of stable transcripts may be the most rapid and efficient means of modulating steady-state mRNA levels and gene expression. Posttranscriptional control of eNOS-mRNA is largely mediated by *cis*-acting RNA elements located in 3' — mRNA untranslated regions (UTRs). Bovine eNOS-mRNA is stabilized by deletion of a 45-nt located at the origin of the bovine 3'-UTR (Searles et al., 1999). A CU-rich 158-nucleotide sequence, located in the medial portion of human 3'-UTR is important for regulating eNOS-mRNA stability (Lai et al., 2003). Another mechanism for post-transcriptional regulation of eNOS has been proposed, based on the evidence of an antisense mRNA (sONE) that is derived from a transcription unit (NOS3AS) on the opposite DNA strand from which the human eNOS (*NOS3*) mRNA is transcribed at human chromosome 7q36 (Robb et al., 2004). The mRNA for sONE can be detected in a variety of cell types, both *in vivo* and *in vitro*, but not in vascular endothelial cells. Suppression of sONE leads to over expression of eNOS whereas over-expression of sONE in human endothelial cells leads to decreased eNOS expression (Robb et al., 2004). These findings suggest a model for cell-specific expression of eNOS that involves a functional interaction between the eNOS and NOS3AS genes at the posttranscriptional level. Further studies are needed to determine how sONE expression is regulated in endothelial cells and its role in eNOS expression under various physiological and pathophysiological conditions.

Endothelial NOS regulation through sub-cellular targeting

A number of variables determine endothelial NO generation and its physiological action, such as the availability of substrates and cofactors, presence of NO scavengers etc. A second layer of regulation exists via the subcellular compartmentalization of eNOS. eNOS is mostly targeted to caveolae in the plasma membrane, where its activity is highest compared to golgi, cytoskeletal and actin associated forms (Oess et al., 2006). In recent years, a picture of complex and precise regulation of eNOS activity involving multi-site phosphorylation of specific serine and threonine residues has emerged (Boo et al., 2006; Church and Fulton, 2006; Zhang et al., 2006). Regulation of endothelial NO synthesis by multi-site eNOS phosphorylation occurs in response to a wide variety of humoral, mechanical and pharmacological stimuli. This regulation involves numerous kinases and phosphatases, as well as interactions with other aspects of eNOS regulation such as Ca^{2+} flux and protein-protein interactions. Oxidized low density lipoprotein (oxLDL) displaces eNOS from caveolae by binding to endothelial cell CD36 receptors and by depleting caveolae cholesterol, resulting in the disruption of eNOS activation (Blair et al., 1999; Feron et al., 1999). Thus, lipoproteins have potent effects on eNOS function in caveolae via actions on both membrane cholesterol homeostasis and on the level of activation of the enzyme. These processes are critically involved in the earliest phases of atherogenesis. Monocrotaline pyrrole, a compound used for developing experimental pulmonary hypertension (Schultze and Roth, 1998), produces golgi blockade in bovine pulmonary aortic endothelial cells, leading to sequestration of eNOS away from its functional caveolar location and providing a mechanism for the reduction in pulmonary arterial NO levels in experimental pulmonary hypertension, despite sustained eNOS protein levels (Mukhopadhyay et al., 2007). This is a classic example where mis-targeting of eNOS results in alteration of NO synthesis despite normal mRNA and protein levels.

Regulation of NOS activity

Reduced bioavailability of NO is considered as one of the most important factors associated with vascular disease. It is unclear however whether this is a cause or a result of endothelial dysfunction. There are a number of factors which affect the production of NO and the ability of NO to reach or diffuse to its cellular targets. An important aspect of NOS function is the availability of substrates and cofactors. It is highly unlikely that L-arginine can become a rate-limiting factor since the K_m of eNOS is approximately $2.9\mu\text{M}$ (Pollock et al., 1991), while intra-cellular levels of L-arginine are 100 fold higher both in culture cells and *in vivo* (Arnal et al., 1999; Baydoun et al., 1990). Studies conducted *in vitro* and *in vivo* however suggest that L-arginine can influence NO production. L-arginine supplementation partially reverses the impairment of endothelium-dependent vasodilation in response to acetylcholine in hypercholesterolemic patients and animal models (Cooke et al., 1992; Cooke and Tsao, 1994). This unexpected response to L-arginine in spite of a large intracellular excess of L-arginine has been termed “the arginine paradox” (Bode-Boger et al., 2007). The synthesis of NO from L-arginine can be blocked pharmacologically by a variety of arginine analogues. In the cardiovascular system, these inhibitors of NOS can induce vasoconstriction, thrombus formation and atherogenesis (Nava et al., 1995). Two of these inhibitors, NG-mono-methyl-L-arginine (L-NMMA) and asymmetrical dimethylarginine (ADMA) are naturally occurring compounds that circulate in plasma (Vallance and Leiper, 2004). The levels of ADMA are regulated by a dynamic process. It is synthesized by the methylation of arginine within proteins, released by proteolysis, and metabolized to citrulline by the enzymes dimethylarginine dimethylaminohydrolase (DDAH)-1 and -2 (Leiper et al., 1999). A novel functional mutation of DDAH-1 carries a significantly elevated risk for cardiovascular disease and a tendency to develop hypertension (Valkonen et al., 2005). Increased plasma ADMA levels have been described in a number of vascular disorders including hypercholesterolaemia, hypertension and is a strong predictor of the risk for acute coronary events (Vallance and Leiper, 2004).

Tetrahydrobiopterin (BH_4), an essential co-factor for NOS, has profound effects on NOS function, including stabilizing its dimeric structure and facilitating and enhancing binding of L-arginine (Cosentino and Luscher, 1999). Reduced bioavailability of BH_4 results in uncoupling of NOS, leading to superoxide ($\text{O}_2^{\cdot-}$) and H_2O_2 production (Stroes et al., 1998). Endothelial cells isolated from diabetic rats have reduced BH_4 levels and reduced NO production (Meininger et al., 2000). Depletion of BH_4 and increased endothelial superoxide production in diabetic wild-type mice with deficient endothelial function, is prevented by maintenance of BH_4 levels in GCH-Tg diabetic mice, over-expressing GTP-cyclohydrolase, the rate-limiting enzyme for BH_4 biosynthesis (Alp et al., 2003). Similarly, in spontaneously hypertensive rats, BH_4 supplementation improves endothelial dysfunction (Heitzer et al., 2000; Hong et al., 2001). Oral administration of BH_4 shows promise for the treatment of oxidative stress-induced disorders, such as the metabolic syndrome (Wang et al., 2007) and improves endothelium dependent vascular relaxation after 10 weeks of high-cholesterol diet (Hattori et al., 2007). Thus, BH_4 represents a therapeutically relevant tool to modulate NOS function in different diseases that are characterized by reduced NOS activity or NO synthesis.

All mammalian cells including endothelial cells generate superoxide anions ($\text{O}_2^{\cdot-}$), which are inactivated mainly by superoxide dismutases (SOD) (Rubbo et al., 1996). The foremost mechanism for the loss of bioavailable NO is thought to be due to its interaction with superoxide. If levels of superoxide increase significantly, NO outcompetes SOD for $\text{O}_2^{\cdot-}$, a reaction which is diffusion-limited for NO and approximately six times faster than dismutation of ($\text{O}_2^{\cdot-}$) by SOD (Beckman and Koppenol, 1996). This reaction has the triple effect of scavenging NO, reducing its bioavailability and producing a potent oxidant, peroxynitrite (ONOO^-). Once formed, peroxynitrite can chemically modify amino acids, nucleic acids and thiol containing proteins and peptides (Koppenol et al., 1992). At physiological pH of 7.4, 20%

of peroxynitrite is protonated to form peroxynitrous acid (ONOOH), which decomposes to form nitrogen dioxide radical ($\text{NO}_2^\cdot$) and hydroxyl radical ($\text{OH}^\cdot$) (Beckman and Koppenol, 1996). The $\text{NO}_2^\cdot$ attacks phenol groups to produce nitrophenols (Ischiropoulos et al., 1992). In biological systems this leads to modification of tyrosine residues to produce 3-nitrotyrosine. The formation of 3-nitrotyrosine can be thought of as a stable biological marker for the formation of peroxynitrite, and is elevated in a number of cardiovascular diseases (Greenacre and Ischiropoulos, 2001; Peluffo and Radi, 2007). Endothelial cells constantly produce low levels of $\text{O}_2^{\cdot-}$, which are significantly increased when the cells become activated (Matsubara and Ziff, 1986). Many vascular diseases are associated with increased superoxide formation. The enzymatic origin of $\text{O}_2^{\cdot-}$ may vary in different types of disease and could potentially involve NAD(P)H oxidases, xanthine oxidase, lipoxygenase and NOS. However both animal and human studies suggest that the primary enzymes responsible for $\text{O}_2^{\cdot-}$ production in the vasculature are the NAD(P)H oxidases (Clemens and Griendling, 2006; Ferder et al., 2006; Inoguchi and Nawata, 2005; Schulman et al., 2006). Hence, a dysfunctional endothelium can contribute to the reduced bioavailability of NO by releasing $\text{O}_2^{\cdot-}$.

Heat shock protein 90 (hsp90), an abundant molecular chaperone (constituting almost 1~2% of total cytosolic protein) is highly conserved from prokaryotes to eukaryotes, and is involved in the folding, stability and maturation of numerous client proteins including nitric oxide synthases (Richter and Buchner, 2001). The hsp90 chaperone machinery is in a constant flux between two conformations. The ADP bound hsp90, which corresponds to an “open” conformation, binds to its client proteins with the assistance of different co-chaperones. Replacement of ADP by ATP results in transient association of the N-terminal domains giving rise to a “closed” structural conformation, which now effectively clamps the client protein and aids in its proper folding, stabilization and maturation (Chadli et al., 2000). Hsp90 inhibitors such as geldanamycin and radicicol, interact with the “N-terminal ATP binding site” of hsp90 and result in destabilization and degradation of the client proteins (Prodromou and Pearl, 2003). Geldanamycin-bound hsp90 resembles the chaperone’s ADP-bound “open” conformation, and this results in the recruitment of other hsp90-interacting proteins such as E3 ubiquitin ligases (e.g. CHIP) which interact with hsp90 and promote ubiquitylation and subsequent proteasomal degradation of client proteins (Marcu et al., 2000).

Endothelial nitric oxide and atherosclerosis

Atherosclerosis is now regarded as the underlying pathology of cardiovascular diseases, such as peripheral vascular disease, stroke and coronary heart disease. The pathology of atherosclerosis is very complex and involves structural elements of the arterial wall, platelets, leukocytes and inflammatory cells, such as monocytes and macrophages. The endothelium forms the dynamic interface between the arterial wall and the afore-mentioned circulating cells. Endothelial dysfunction thus constitutes one of the primary causes of initiation of atherosclerosis. Since the endothelium is a major source of NO in the vasculature, loss of normal cellular function would result in altered eNOS function and NO synthesis. The endothelium has a constitutive supply of NO from eNOS and under certain conditions as inflammation, can produce excessive NO from the inducible isoform of nitric oxide synthase or iNOS (MacNaul and Hutchinson, 1993). Regulation of nitric oxide synthases and bioavailability of their product therefore become critical for the development and progression of vascular diseases, such as atherosclerosis. Several lines of evidence indicate that endothelium-dependent vascular relaxation is impaired in cholesterol-fed animals (Kojda et al., 1998) or in isolated human coronary arteries (Forstermann et al., 1988) and the impairment is correlated with the degree of atherosclerosis (Otsuji et al., 1995). Administration of L-arginine (Aji et al., 1997; Boger et al., 1997) or tetrahydrobiopterin (Hattori et al., 2007; Tiefenbacher et al., 2000) attenuates atherosclerotic lesion progression, whereas administration of NOS inhibitors block this protective effect (Wang et al., 1996), signifying a direct link between NO

and atherosclerosis lesion formation. NO has also been shown in various *in vitro* and *in vivo* studies to exert anti-inflammatory effects, such as inhibition of endothelial adhesion molecule (VCAM-1, ICAM-1) and tissue factor (TF) expression (Kubes et al., 1991) and inhibition of release of chemokines, such as monocyte chemoattractant protein-1 or MCP-1 (Zeiher et al., 1995). Moreover, NO blocks platelet aggregation and possesses fibrinolytic effects (Cooke and Dzau, 1997). However, endothelial NO can play a dual role in atherosclerosis. High levels of NO produced from iNOS in endothelial cells and macrophages can induce injury to the endothelium. Peroxynitrite (ONOO⁻), the product of NO interaction with superoxide, is produced in significant amounts in atherosclerotic lesions. Peroxynitrite can oxidize BH₄ (Kohnen et al., 2001) and reduce physiological levels necessary for eNOS function, shifting eNOS from an NO generating enzyme to a superoxide producing enzyme (Xu et al., 2006). Thus NO can have both anti- and pro-atherosclerotic effects based on the course of disease progression. Clinical trials directed towards oral dosage of L-arginine have given mixed results (Adams et al., 1997; Blum et al., 2000) with one study that showed no indication of improvement of mortality (Oomen et al., 2000). Hsp90-inhibitors could prove to be beneficial with respect to some of the pro-inflammatory mediators that require hsp90 for plaque formation in atherosclerosis. Notably, matrix-metalloproteinase-2, that has been shown to play a role in plaque formation (Kuzuya et al., 2006), requires hsp90 for its proper folding and function (Eustace and Jay, 2004). Macrophage scavenger receptors (MSR), that engulf oxidized-LDL and result in foam cell and plaque formation, associate with hsp90 signifying a possible link between hsp90 in MSR function (Nakamura et al., 2002). Since NO is thought to play a detrimental role in later stages of atherosclerosis, hsp90 inhibitors, by blocking inducible and endothelial nitric oxide synthases, might prove clinically useful by inhibiting NO formation.

Endothelial nitric oxide and diabetes

Diabetes mellitus, which essentially represents a heterogeneous group of disorders that have hyperglycemia as a common feature, is characterized by endothelial dysfunction (McCloud et al., 2004; Rask-Madsen and King, 2007). Human IDDM (insulin dependent diabetes mellitus) and NIDDM (non-insulin dependent diabetes mellitus), and animal models of IDDM are all associated with reduced bioavailability of nitric oxide and impaired endothelium-dependent relaxation (Durante et al., 1988; Johnstone et al., 1993; Williams et al., 1996). As with atherosclerosis, conflicting reports exist regarding altered expression and activity of NOS in diabetic animal models. Under hyperglycemic conditions, human aortic endothelial cells have been shown to have either reduced (Srinivasan et al., 2004) or increased eNOS expression (Cosentino et al., 1997). Human glomerular endothelial cells on the other hand exhibit increased eNOS expression but reduced NO formation under hyperglycemic condition (Hoshiyama et al., 2003). Interestingly, eNOS knockout mice exhibited accelerated diabetic nephropathy (Zhao et al., 2006) supporting a role for deficient eNOS-derived NO production in the pathogenesis of diabetic nephropathy. Other diabetic animal models have shown both increased mRNA and protein for eNOS (Pieper et al., 1997), despite other studies that showed reduced cGMP formation (Lin et al., 2002). Under hyperglycemic conditions, *in vivo* in rats, mesenteric arteries produced reduced NO (Stalker et al., 2003) and exhibit reduced hsp90-eNOS complex formation. Metformin, a bioguanide derivative (dimethylbiguanide) and one of the most commonly used drugs for the treatment of type 2 diabetes (Abbasi et al., 2004), dramatically attenuated high glucose-induced reduction in the association of hsp90 with eNOS in bovine aortic endothelium, resulting in increased NO bioactivity and a reduction in overexpression of adhesion molecules and endothelial apoptosis caused by high glucose exposure (Davis et al., 2006). Thus, in the setting of hyperglycemia, enhancing eNOS activity and NO bioavailability has beneficial effects. It has been shown that heat stress upregulates eNOS expression and endothelial NO release. Whether, this effect is beneficial in the setting of hyperglycemia remains to be investigated. It is crucial to include the importance of iNOS in diabetic pathophysiology since recent reports have revealed decreased expression of

endothelial nitric oxide synthase (eNOS) concomitant with increased expression of iNOS and nitrotyrosine during the progression of diabetes in rats (Nagareddy et al., 2005). This finding suggests that induction of iNOS in cardiovascular tissues is dependent on the duration of diabetes and contributes significantly to the depressed pressor responses to vasoactive agents and potentially to endothelial dysfunction.

Hyperglycemic conditions induce endothelial production of superoxide and synthesis of anti-oxidant enzymes (Ceriello et al., 1996; Graier et al., 1999). Diabetic subjects have reduced anti-oxidant capacity which could favor oxidative stress (Beckman et al., 2002). Peroxynitrite anions are an important source of reactive nitrogen intermediates that nitrate or s-nitrosylate proteins and thereby modify their functions. In animal models of diabetes, impaired endothelium-dependent relaxation was abrogated after acute incubation with superoxide dismutase, a superoxide scavenger (Hattori et al., 1991; Ohishi and Carmines, 1995; Rosen et al., 1995). These results are suggestive of a major role of destruction of NO by $O_2^{\cdot -}$ in diabetes associated vascular dysfunction.

Endothelial nitric oxide and hypertension

Nitric oxide is crucial for maintenance of normal blood pressure (Huang et al., 1995) and therefore the role of NO in essential hypertension has been an area of intense investigation. Impaired NO-mediated vasodilation has been shown in animal models of hypertension (Lockette et al., 1986; Winquist et al., 1984) and hypertensive patients (Higashi et al., 1995; Treasure et al., 1993). It is still not clear whether this is due to reduced synthesis or increased consumption of NO. In patients with arterial hypertension, blood flow responses to infusions of organic nitrates were significantly impaired compared to normotensive controls (Preik et al., 1996). The impairment of dilation of resistance arteries in response to infusion of nitric oxide donors correlated with the severity of arterial hypertension. However, elsewhere, no difference was noted in serum NO(x) levels between normotensive and hypertensive patients without comorbid diseases, such as atherosclerosis or diabetes mellitus (Higashino et al., 2007), and Ca^{2+} dependent NOS activity was reported comparable between aortas of hypertensive and normotensive rats (Nava et al., 1996). Similarly, NO production was normal in spontaneously hypertensive rats, but $O_2^{\cdot -}$ production was elevated and led to increased oxidation of NO, resulting in a decreased vasodilation response (Heitzer et al., 2000). The $O_2^{\cdot -}$ could arise from a variety of sources, including NAD(P)H oxidases and cyclo-oxygenase, since these enzymes are upregulated in hypertension (Taddei et al., 1998; Zalba et al., 2000). Thus, essential hypertension is associated with reduced endothelium-dependent relaxation, where the functional role of NO is regulated through its destruction or modification by another radical, namely superoxide.

Regulation of endothelial NOS is also achieved through protein-protein interactions of eNOS with heat shock protein (hsp)-90 and caveolin-1 among many others. There are excellent reviews that address various aspects of eNOS regulation (Kone, 2000; Sessa, 2004; , 2005). Here, we will focus on recent findings that gave us new information about hsp90-mediated regulation of NO in pathophysiological conditions. Many of the biological effects of NO is mediated through its binding with the heme prosthetic group of soluble guanylate cyclase (sGC) which activates sGC, that catalyzes the conversion of GTP to cGMP. It was shown that sGC, eNOS and hsp90 existed as trimeric complexes in bovine aortic endothelial cells and sGC interactions with eNOS and hsp90 were regulated in an agonist-dependent manner (Venema et al., 2003). Furthermore, SNP-induced decrease in mean arterial pressure in rats is attenuated by intravenous geldanamycin, an hsp90 inhibitor, suggesting that sGC-hsp90 interactions are important regulators of NO-mediated effects. A few studies have looked into hsp90 and eNOS expression and their association in hypertension-associated pathophysiology. Hsp90 overexpression was observed in a case of hypertension-induced renal failure in human renal

tubular cells (Komatsuda et al., 1999). Similarly, in spontaneously hypertensive rats, hsp90 expression was higher in left ventricles and mesenteric arteries but not in aorta compared to normotensive controls. (Piech et al., 2003). Moreover, endothelial hsp90 expression (Ai et al., 2003), NOS activity and NO production (Shah et al., 1999) are increased in mesenteric vascular beds of rats with portal hypertension. Hsp90 overexpression has also been observed in cerebral microvessels of spontaneously hypertensive rats (Zhou et al., 2005). Modulation of hsp90 function through hsp90 inhibitors in these disease settings has not been investigated thoroughly.

Endothelial nitric oxide and angiogenesis

Angiogenesis is a tightly regulated physiological process (e.g. in female reproductive tract during normal reproductive cycles, wound healing etc.) that leads to the formation of new blood vessels from preexisting ones (Liekens et al., 2001). Angiogenesis also occurs in pathological situations, such as retinopathies, arthritis, endometriosis and cancer (Walsh, 2007). Enhanced angiogenesis is present in tumors that need new blood capillaries to grow, remove metabolic waste and transport the cells to locations distal to the primary tumor, facilitating metastasis (Folkman, 1971). For these reasons, blockade of angiogenesis is an attractive approach for the treatment of both solid and haematological malignancies. It has been shown that hsp90-eNOS interaction is important for mediating the angiogenic response (Brouet et al., 2001). The proangiogenic effects of statins are dependent on eNOS-mediated NO production, and are geldanamycin-sensitive. The importance of hsp90-eNOS interaction has been shown in other models of angiogenesis (Beliakoff and Whitesell, 2004; Bergstrom et al., 2006; de Candia et al., 2003; Kurebayashi et al., 2001; Sun and Liao, 2004), which indicate that this ubiquitous chaperone plays a crucial role in regulating NO-mediated effects in angiogenesis.

Endothelial nitric oxide and apoptosis

The availability of intracellular arginine is a rate-limiting factor in NO production. Low concentrations of NO protect cells from apoptosis whereas higher concentrations, as seen in cerebral infarction, sepsis, diabetes mellitus etc., cause apoptosis. Both NOS and arginase use arginine as a common substrate, and arginase may down-regulate NO production by competing with NOS for arginine. Both arginase I and arginase II as well as iNOS are induced in LPS-activated mouse peritoneal macrophages (Louis et al., 1998; Salimuddin et al., 1999). The co-expression of arginase with iNOS therefore serves as a mechanism for preventing overproduction of NO because overproduction of NO is toxic to macrophages and neighboring cells. LPS induces both iNOS and arginase II but not arginase I in rat aortic endothelial cells (Bugu et al., 1996). Arginase inhibition enhances NO production and dilation in normal vessels and restores NO-mediated dilation after ischemia-reperfusion (Hein et al., 2003). Arginase also reduces NO production in aortic rings from aging rats (Berkowitz et al., 2003). Therefore, arginase down-regulates NO production and may have important implications for cardiovascular function. It has been suggested by several studies that apoptosis is important for the pathogenesis of atherosclerosis (de Nigris et al., 2003; Kutuk and Basaga, 2006). Anti-oxidants protect bovine aortic endothelial cells from doxorubicin-induced apoptosis, through up-regulation of hsp70, an anti-apoptotic protein (Kalivendi et al., 2001). Hsp70 has been reported to act in some situations upstream or downstream of caspase activation, and its protective effects may be either dependent on or independent of its ability to inhibit JNK activation (Mosser et al., 2000). Preconditioning of bovine aortic endothelial cells with small doses of NO, protects them from apoptosis induced by higher dose of NO, an effect that was lost when cells were pretreated with an hsp90 or sGC inhibitor (Antonova et al., 2007). ACE inhibitors, that cause bradykinin receptor mediated eNOS activation and NO production, exert a protective effect on atherosclerosis lesion formation in baboons (Linz et al., 1995).

Modulation of NO-cGMP signaling through hsp90-sGC association therefore constitutes an exciting area in apoptosis research.

Endothelial nitric oxide and sepsis

Sepsis and septic shock are associated with overproduction of nitric oxide primarily through iNOS (Titheradge, 1999). The endothelium is a key player in initiating, perpetuating, and modulating the host response to infection. Role of eNOS in the pathophysiology of sepsis has recently gained controversy due to findings that indicated eNOS as a pro-inflammatory candidate in inflammatory disease conditions. Chronic eNOS over-expression in the endothelium of transgenic mice resulted in resistance to LPS-induced hypotension, lung injury, and death (Yamashita et al., 2000) whereas, in another study, tissue (heart, liver, lungs, aorta) iNOS expression was greatly reduced in eNOS knockout mice after LPS injection with an improved hemodynamic profile. This and other *in vitro* (Connelly et al., 2003) and *in vivo* (Bucci et al., 2005) studies highlight a pro-inflammatory role of eNOS in inflammatory diseases. We have shown that pretreating mice with hsp90 inhibitors markedly improves survival and lung function following a lethal dose of LPS and is associated with reduced lung injury and formation of hsp90-iNOS complexes and NO metabolites (Chatterjee et al., 2007). Our preliminary experiments also revealed reduced pulmonary eNOS expression in septic mice, pretreated with hsp90 inhibitors (unpublished data). Therefore, in our model, hsp90 appears to exert a pro-inflammatory role through its association with eNOS and iNOS. How hsp90 regulates endothelial NO post sepsis remains an interesting topic to be investigated.

Concluding remarks

Regulation of NO and NO-mediated effects can occur through multiple mechanisms, including targeting of eNOS, availability of factors that regulate NOS activity or through modulation of protein-protein interactions of NOS with its partners, most importantly, hsp90. The complex response of endothelial cells to pathophysiological stimuli among different vascular beds or among different animal models of complex vascular disorders, such as diabetes and sepsis is a reflection of the complexity of NO regulation in endothelial cells. Although the role of tetrahydrobiopterin, reactive oxygen species, and hsp90, among many others, in regulating NO biological activity are well established, a more thorough understanding of their role is required to translate these findings into successful clinical therapies

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