MINIREVIEW

eNOS Function and Dysfunction in the Penis

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Endothelial nitric oxide (NO) synthase (eNOS) has an indispensable role in the erectile response. In the penis, eNOS activity and endothelial NO bioavailability are regulated by multiple post-translational molecular mechanisms, such as eNOS phosphorylation, eNOS interaction with regulatory proteins and contractile pathways, and actions of reactive oxygen species (ROS). These mechanisms regulate eNOS-mediated responses under physiologic circumstances and provide various mechanisms whereby endothelial NO availability may be altered in states of vasculogenic erectile dysfunction (ED). In view of the recent advances in the field of eNOS function in the penis and its role in penile erection, the emphasis in this review is placed on the mechanisms regulating eNOS activity and its interaction with the RhoA/Rho-kinase pathway in the physiology of penile erection and the pathophysiology of ED. Exp Biol Med 231:154–165, 2006

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Introduction

Penile erection is a complex neurovascular process involving relaxation of the corpus cavernosal smooth muscle combined with increased arterial inflow into the penis and restricted venous outflow from the organ. The nerves, endothelium of sinusoids and blood vessels, and smooth muscle cells in the penis produce transmitters and modulators that control the erect versus flaccid state of the penis.

The nitric oxide (NO) pathway is of critical importance in the physiologic induction and maintenance of erections (1, 2). The constitutive endothelial NO synthase (eNOS) and neuronal NO synthase (nNOS) isoforms are tightly regulated and produce physiologically relevant levels of NO in endothelial cells and autonomic nerve endings of the penis. Although neurally derived NO is well established as a mediator of penile erection (2), the role of eNOS in penile erection is becoming increasingly recognized. Recent advances in the field of penile vascular biology and the regulatory biology of penile erection indicate that the regulation of eNOS in the penis involves multiple molecular mechanisms that act in concert to both positively and negatively affect the function of this enzyme.

Endothelial NO is an important mediator of both physiologic and pathologic responses in the penis. In normal endothelial function, NO has vasodilatory properties and counterbalances RhoA/Rho-kinase–mediated vasoconstriction, thus, regulating vascular tissue homeostasis. Conversely, during pathologic conditions, eNOS uncoupling and formation of peroxynitrite from the reaction of NO with superoxide anion results in pro-oxidant effects of NO. The balance between NO bioavailability, vasoconstrictor function, and vascular generation of reactive oxygen species (ROS) is crucial for maintaining normal erectile ability.

Regulation of eNOS Activity and Bioavailability

eNOS is controlled through its transcriptional regulation and post-translationally through the regulation of its activity. The latter mechanisms involve calcium/calmodulin binding; fatty acid modification (such as myristoylation and palmitoylation); alterations in intracellular translocation, substrate, and cofactor availability; dimerization of the enzyme subunits; binding to other proteins/cofactors (such as caveolin-1 and heat-shock protein [Hsp]-90); and

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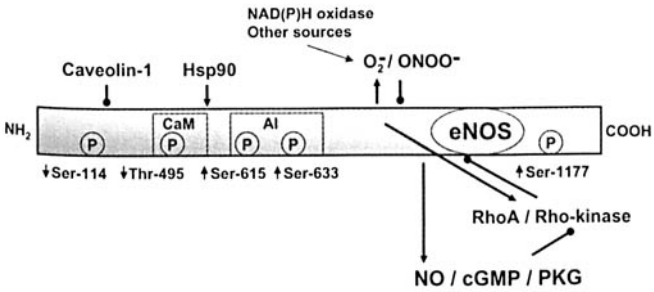

Figure 1. Major regulators of eNOS activity and endothelial NO bioavailability. Phosphorylation of eNOS at different sites results in increased (↑) or decreased (↓) enzyme activity. Interaction of Hsp90 with eNOS positively regulates the enzyme's activity, whereas interaction of eNOS with caveolin-1 and peroxynitrite ($ONOO^-$) negatively regulates the enzyme's activity. Superoxide (O_2^-) produced by NAD(P)H oxidase and other sources may uncouple eNOS through the formation of $ONOO^-$, which may further increase O_2^- production and activate the RhoA/Rho-kinase pathway. A negative functional interrelationship exists between eNOS and the RhoA/Rho-kinase pathway, whereby each pathway may inhibit the other. eNOS activation results in NO/soluble guanylyl cyclase/cGMP/protein kinase G (PKG)-dependent relaxation of vascular smooth muscle. (–•) denotes inhibitory effect.

phosphorylation. Figure 1 summarizes the major regulators of eNOS activity and NO bioavailability.

Positive Regulators of eNOS Activity. *Calcium/Calmodulin.* The activation of eNOS in response to agonists such as acetylcholine or bradykinin 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 and displaces an adjacent auto-inhibitory loop, promoting nicotinamide adenine dinucleotide phosphate (NADPH)-dependent electron flux from the C-terminal reductase domain of one monomer to the N-terminal oxygenase domain of the other monomer (3). This classic mode of eNOS activation by calcium/calmodulin accounts for rapid and transient production of endothelial NO, as has been well documented in the penis.

Phosphorylation of eNOS at Positive Regulatory Sites. eNOS can be phosphorylated by several stimuli in the absence of a sustained increase in intracellular calcium. The most important physiologic agonist for such eNOS activation 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, increase both phosphatidylinositol 3-kinase (PI3-K)/Akt– and protein kinase A–dependent constitutive endothelial NO production after phosphorylation of the endogenous eNOS at Ser-1177 (human sequence, equivalent to Ser-1179 of bovine eNOS) by reducing the enzyme's calcium requirement and facilitating electron transfer (4–8). Depending on the cellular context and a given stimulus, eNOS (Ser-1177) can be phosphorylated by other protein kinases in addition to Akt and PKA, including AMP-activated protein kinase, cAMP- and cGMP-dependent protein kinases, calmodulin II protein kinase, and protein kinase C (PKC; Ref. 9).

PI3-K/Akt–dependent eNOS activation has recently been shown to be operative in the penis. In rats and mice, both neuro- and agonist-induced penile erection produces rapid increases in phosphorylated Akt and phosphorylated eNOS at Ser-1177 in the penis, which remain elevated after the termination of the initial stimulus (10). In addition to blood flow–related shear stress, vascular endothelial growth factor (VEGF) also activates eNOS in the penis by phosphorylation at Ser-1177, but this effect seems to be independent of Akt activation (11).

In addition to Ser-1177, eNOS can be phosphorylated at several other sites, including Ser-114, Thr-495, Ser-615, and Ser-633 (human sequence, equivalent to Ser-116, Thr-497, Ser-617, Ser-635 of bovine eNOS), which increase or decrease the enzyme's activity directly or by modulating other regulatory sites on eNOS. Ser-633 and Ser-615 phosphorylation increases the enzyme's activity (7, 12, 13). The roles of these phosphorylation sites on eNOS in penile erection have only been preliminarily evaluated, as discussed below in "Diabetes Mellitus."

Trafficking Proteins That Stimulate eNOS Function. The chaperone protein Hsp90 can interact with eNOS and positively influence its function. The interaction of Hsp90 with eNOS is stimulated by agonists, such as shear stress, VEGF, and estrogen. The increase in eNOS phosphorylation after Hsp90 is recruited to eNOS is achieved through reduced dephosphorylation of Akt, increased ability of Akt to phosphorylate Hsp90-bound eNOS, and calmodulin-dependent disruption of eNOS binding with caveolin-1 (14, 15). The interaction between the complex of eNOS with Hsp90 and Akt is also regulated by eNOS phosphorylation on the Ser-615 and Ser-114 residues (7). Hsp90 has also been implicated in the balance between the production of NO and superoxide by eNOS (16).

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).

The role of positive eNOS protein modulators in penile erection has only started to be investigated. Our preliminary studies show that Hsp90 and eNOS colocalize in the rat penis, but the functional significance of this interaction has not yet been evaluated.[1]

Negative Regulators of eNOS Activity. *Phosphorylation of eNOS at Negative Regulatory Sites.* Phosphorylation of the Thr-495 residue on eNOS, most probably by PKC, is associated with a decrease in eNOS activity by increasing the calcium/calmodulin dependence of the enzyme. Reciprocal dephosphorylation of Thr-495 and phosphorylation of Ser-1177 seem to be

[1] Musicki B, Liu T, Burnett AL. 2005. Unpublished data.

essential for eNOS activity (17–19). The phosphorylation of Ser-116 also seems to reduce eNOS function (20).

The roles of these phosphorylation sites on eNOS in penile erection have only been preliminarily evaluated. In the rat penis, electrical stimulation of the cavernous nerve increases phosphorylation of eNOS at Thr-495 (21), suggesting a role of eNOS in preventing its own overactivation, which would cause an excessive eNOS-dependent erection. Mitogen-activated protein (MAP) kinases 1 and 2 (extracellular signal-regulated protein kinase [ERK] 1/2) also negatively regulate eNOS activity. Sommer *et al.* demonstrated that the expression of ERK 1/2 is substantially greater in the corporal smooth muscle of men with ED associated with various primary diseases compared with potent men (22). The mechanism of eNOS inhibition by ERK1/2 in the penis is not known.

Trafficking Proteins That Inhibit eNOS Function. eNOS is a membrane-associated NOS isoform. The majority of eNOS is bound to caveolin-1 in caveolae, and the enzyme activity of eNOS is basally repressed (23). Caveolal localization of eNOS is mediated by its co-translational N-myristoylation and post-translational cysteine palmitoylation (23). Compartmentalization of eNOS in caveolae is necessary for its interaction with regulatory proteins and calcium- and phosphorylation-dependent signal transduction events that modify the response of the enzyme to extracellular stimuli. Caveolae are specialized cholesterol-rich domains that compartmentalize signal transduction molecules (23, 24). Stimuli, such as shear stress, induce calcium increase, and the calcium/calmodulin complex displaces eNOS from caveolin-1 and leads to the redistribution of eNOS from plasma membrane caveolae and away from its tonic inhibition (24, 25).

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).

Little information is available regarding subcellular localization of eNOS and its interaction with negative protein modulators in the penis and its physiologic significance with respect to penile erection *in vivo*. Our preliminary data show that eNOS colocalizes with caveolin-1 in the penis. Electrical stimulation of the cavernous nerve decreases the amount of caveolin-1 associated with eNOS. On the contrary, increasing the interaction between eNOS and caveolin-1 by intracavernosal injection of AP-Cav peptide, a caveolin surrogate that binds to eNOS (26), decreases the erectile response, suggesting that the eNOS and caveolin-1 interaction diminishes eNOS activation in the penis and accordingly opposes eNOS-mediated penile erection.[2]

[2] Musicki B, Liu T, Burnett AL. 2005. Unpublished data.

eNOS Autoinhibitory Element. Another mechanism that accounts for the tonic inhibition of eNOS is the presence of an autoinhibitory domain in the reductase domain of eNOS (residues 604–643 based on the bovine sequence) that physically impedes the binding of calcium/calmodulin to eNOS (27). eNOS phosphorylation residues Ser-617 and Ser-635 are also within this site. Calcium/calmodulin binding releases the autoinhibitory control element.

G-Protein–Coupled Receptors. Several G-protein–coupled receptors bind to eNOS and inhibit the enzyme. The bradykinin B2 receptor, the angiotensin II receptor, and the endothelin-1 receptor reduce eNOS activity (28). Activation of the B2 receptors by bradykinin in human erectile tissues leads to a dissociation of the eNOS-B2 receptor complex, releasing eNOS, and resulting in the relaxation of the corpus cavernosum (29).

RhoA/Rho-Kinase Pathway. Contraction of smooth muscle is primarily mediated by calcium-dependent activation of myosin light chain kinase, resulting in phosphorylation of myosin light chain and actin/myosin assembly. The calcium-independent increase in vascular smooth muscle tone, known as calcium-sensitization, is largely mediated by activation of the small GTPase, RhoA, and its downstream effector, Rho-kinase (30). RhoA may be activated by several signaling pathways, including the binding of G-protein–coupled receptor agonists. RhoA-activated Rho-kinase (α and β isoforms) phosphorylates and inhibits regulatory myosin phosphatase target subunit 1 (MYPT1) of myosin light chain phosphatase at Thr-696 and inhibits its activity, promoting smooth muscle contraction (31). An inverse functional relationship exists between the NO/cGMP/protein kinase G and RhoA/Rho-kinase signaling pathways within the vasculature. The NO pathway phosphorylates RhoA at Ser-188, which prevents its translocation to the membrane and activation (32). In addition, in human endothelial cells, the RhoA/Rho-kinase pathway inhibits Akt-dependent eNOS activity/phosphorylation at Ser-1177 (33), providing an additional means of interaction between eNOS-mediated relaxant and RhoA/Rho-kinase–mediated contractile pathways.

The degree of contraction of the corpus cavernosum smooth muscle and the functional state of the penis is determined by the balance between proerectile and anti-erectile mechanisms that operate physiologically in the penis. Vasoconstriction (evoked by norepinephrine through α-adrenergic receptors, endothelins, angiotensins, and thromboxane A_2), which maintains the penis in the flaccid state, is mediated, in part, by the RhoA/Rho-kinase pathway (34–36). During erection, this pathway is inhibited, most likely by NO (37). In addition, RhoA/Rho-kinase suppresses eNOS gene expression and enzyme activity in the penis (38). The selective Rho-kinase inhibitors Y-27632 and H-1152 (34, 39, 40) and adeno-associated viral gene transfer of dominant negative RhoA to the penis (41) enhance erectile function in rats. Figure 2 schematically depicts regulation of

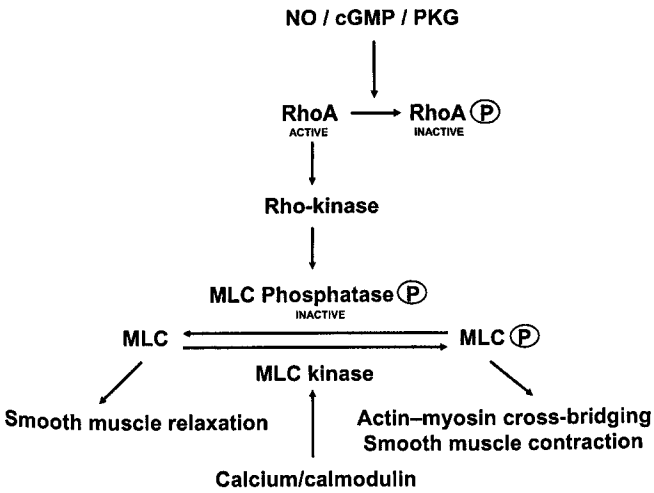

Figure 2. Regulation of smooth-muscle tone; relaxation by NO versus contraction by RhoA/Rho-kinase pathways. After vasoconstrictor-mediated increases in intracellular calcium levels, calcium/calmodulin activates myosin light chain (MLC) kinase leading to increased phosphorylation of MLC and contraction. Calcium-sensitization involves the RhoA/Rho-kinase system. Activated RhoA activates Rho-kinase, which phosphorylates MLC phosphatase (MLC Phosphatase-P), inhibiting its activity. Inhibition of MLC phosphatase increases MLC phosphorylation (MLC-P), which promotes the actin-myosin cross bridge cycling rate, resulting in smooth muscle contraction. NO/cGMP/protein kinase G (PKG) phosphorylates RhoA, which prevents its translocation to the membrane and activation. Inactive RhoA prevents Rho-kinase mediated contraction and results in smooth muscle relaxation.

the contractile pathway in smooth muscle and its interaction with the NO relaxant pathway in the penis.

ROS. Both eNOS activity and endothelial NO availability are subject to regulation by ROS. Endothelial dysfunction, which primarily reflects decreased availability of endothelial NO, arises, in part, because of increased production of ROS, specifically of superoxide anion. Superoxide anion is produced in a variety of cells, including vascular smooth muscle cells and endothelial cells. Potential vascular sources of superoxide anion include, among others, uncoupled eNOS, xanthine oxidase, NAD(P)H oxidase, and mitochondrial electron transport (42). Superoxide anion may directly inactivate NO and decrease its bioavailability. Moreover, the reaction of superoxide anion and NO results in the formation of the highly toxic molecule, peroxynitrite (43). Peroxynitrite may cause oxidative damage to DNA, proteins, and lipids; promote release of vasoconstrictors; increase apoptosis; and cause tissue injury. It may also oxidize eNOS, resulting in eNOS uncoupling. Oxidation of the zinc thiolate cluster of eNOS (destabilizing the eNOS dimer) and the cofactor tetrahydrobiopterin (BH4) by peroxynitrite, and the decreased availability of the NOS substrate L-arginine, lead to a switch from an NO-producing to a superoxide-producing eNOS, a phenomenon termed eNOS uncoupling (43). eNOS uncoupling is not only associated with increased superoxide anion formation, which reacts with NO, further generating peroxynitrite, but also reduced NO production, because the electron flux is shifted from L-arginine to molecular oxygen. Enzymes that degrade ROS, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, play an important role in the cellular protection against ROS. SOD accelerates the dismutation of superoxide into hydrogen peroxide and molecular oxygen. Three SOD isoforms have been identified: a cytosolic copper/zinc–containing form, a mitochondrial manganese form, and an extracellular isoform. Endothelial and smooth muscle cells of the penis contain cytosolic copper/zinc and extracellular isoforms of SOD (44). The imbalance between the production and elimination of ROS, a condition known as oxidative stress, has an important role in the development of endothelial dysfunction and ED in systemic vascular disorders, such as diabetes, hypercholesterolemia, hypertension, and aging.

In addition to its effect on eNOS and endothelial NO, ROS also affect RhoA/Rho-kinase signaling. In nonpenile vascular beds, ROS have been shown to activate the RhoA/Rho-kinase pathway by promoting increased migration of RhoA to the plasma membrane and its activation (45), thus, stimulating vasoconstriction.

eNOS uncoupling and rapid inactivation of NO by ROS may offer an explanation for seemingly contradictory findings of compensatory upregulation of eNOS expression in different vascular beds, including the penis, associated with several forms of vasculogenic ED, as discussed at the end of the review in "Complexity of eNOS Function in the Penis."

eNOS in Penile Erection

The classic concept of nNOS and eNOS activation by calcium/calmodulin accounts for the rapid and transient production of NO. As such, calcium-dependent mechanisms initiate penile erection, whereas calcium-"independent" production of endothelial NO is responsible for the achievement and maintenance of full erection, an event which lasts longer than a transient and short-term increase in intracellular calcium concentrations. In response to sexual stimuli, vasorelaxation from neurally derived NO initiates erection by causing rapid and short-term increases in penile blood flow and physical expansion of penile vasculature and sinusoidal spaces. The resulting shear force on the endothelium of these structures activates PI3-K/Akt/eNOS (Ser-1177) phosphorylation, causing sustained NO release (Fig. 3). Consistent with this model, NO production persists in the penis much longer than the initial stimulus, enabling continued relaxation and full erection (10).

Vasculogenic ED

ED is the consistent inability to achieve and maintain penile erection sufficient for adequate sexual relations. It is a prevalent condition affecting more than 150 million men worldwide, and is predicted to double within the next 20 years (46).

ED is predominantly a vascular disease. A variety of

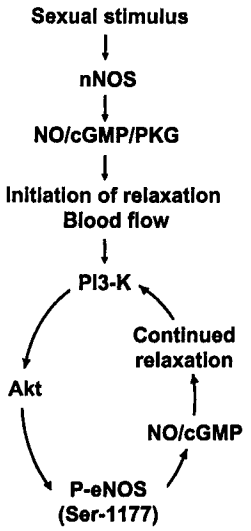

Figure 3. Integrative roles of calcium-dependent activation of nNOS in the initiation of penile erection, and calcium-"independent," shear stress–mediated activation of eNOS in the maintenance of penile erection.

conditions that involve vascular abnormalities, such as diabetes, aging, hypercholesterolemia, hypertension, sedentary life style, and cigarette smoking, are associated with the impairment of penile vascular function and vasculogenic ED in men and in a number of animal models. Mechanisms that may be related to a loss in endothelial NO bioavailability in the penis include decreased eNOS expression and activity, dysregulation of eNOS phosphorylation, increased NO scavenging by ROS or oxidized low-density lipoprotein (LDL), eNOS uncoupling, decreased levels of eNOS cofactors and substrate, impaired interaction of eNOS with its regulatory proteins, and increased interaction with a contractile signaling pathway.

The following subsections pertain to specific molecular mechanisms involving eNOS in the penis for several prominent forms of vasculogenic ED.

Aging. The incidence of ED increases with age. Erectile impairment with aging has been attributed to a reduction in nonadrenergic noncholinergic nerve fibers in the penis, decreased endothelium- and neurogenic-mediated corpus cavernosum relaxation, reduction in NO biosynthesis caused by decreased NOS expression, decreased eNOS activity, increased oxidative stress, impaired eNOS phosphorylation, and increased expression/activity of the RhoA/Rho-kinase pathway (47–52). In addition, reduced activation of protein kinase G-I by cGMP (53) and increased expression of phosphodiesterase-5 (54), which hydrolyzes cGMP, has also been described in the aged penis.

Impaired eNOS expression and activity in the penis of aged rats may be restored with *in vivo* adenoviral gene transfer of eNOS to the aged penis. eNOS overexpression increases eNOS protein level and activity, elevates cGMP levels, and restores erectile responses without affecting nNOS expression (52). Another mechanism that accounts for downregulated NO production in penes from old animals relates to decreased content of the NOS substrate, L-arginine, and increased activity of arginase, an enzyme that competes with eNOS for the common substrate, L-arginine. Impaired endothelium-dependent relaxation of the aged penis may be normalized in the presence of an arginase inhibitor or by the supplementation of excess L-arginine (55, 56).

Oxidative stress is an important factor contributing to endothelial dysfunction associated with aging. The production of superoxide anion is increased in a number of vascular beds with advanced age. The association of oxidative stress in the penis and age-related ED has only recently been investigated. Penes from old rats exhibit an increase in nitrotyrosine immunostaining, a specific marker of peroxynitrite formation (44, 57). Endothelium and corpus cavernosal smooth muscle of the aged rat penis produce high levels of superoxide anion compared with those of younger animals, without a concomitant increase in SOD to detoxify it. Accordingly, gene transfer of extracellular SOD to the penis of aged rats reduces superoxide anion formation and restores erectile function (44).

Another mechanism underlying age-related ED is associated with eNOS inactivation through dysregulation of its phosphorylation. Akt-dependent phosphorylation of a positive regulatory site (Ser-1177) on eNOS is decreased, whereas phosphorylation of a negative regulatory site (Thr-495) on eNOS is increased in the aged rat penis. Phosphorylation of the Ser-1177 residue on eNOS in the aged rat penis is also decreased in response to shear stress elicited by electrical stimulation of the cavernous nerve, presumably because of increased phosphorylation of Thr-495 on eNOS, which prevents Akt-dependent phosphorylation of Ser-1177 (21).

In addition to decreased activity of eNOS and bioavailability of NO, the impairment of corpus cavernosum relaxation with aging is also caused by an increased release or activity of vasoconstrictors, which increase contractile tone in the penile vasculature. RNA and protein levels of endothelin-1 are increased in the penis of aging Brown Norway rats (58). The activity of Rho-kinase is elevated in the aged rat penis (54), whereas inhibition of the RhoA/Rho-kinase pathway with a specific inhibitor, Y-27632, improves the erectile response in old rats (59).

Diabetes Mellitus. Diabetes mellitus is one of the major risk factors for ED. The incidence of ED is higher in diabetic than in age-matched nondiabetic men, and this difference increases with age. It has been estimated that 50%–75% of diabetic men have ED to some degree (60).

Hyperglycemia is thought to contribute to many vascular complications and metabolic derangements associated with both Type I and Type II diabetes, although the majority of studies apply to Type I diabetes. Diabetes-associated ED has been attributed to a reduction in the number of NOS-containing nerves, the impairment of NOS activity, and both neurogenic- and endothelium-mediated

smooth muscle relaxation (61–63), and also to down-regulation of the mediators downstream from NO, such as cGMP (64) and cGMP-dependent protein kinase-1 (65), in the corpus cavernosum. Several mechanisms of impaired endothelial function in the diabetic penis have been described that contribute to vasculogenic ED. They include reduced eNOS expression, decreased eNOS activity and impaired eNOS phosphorylation, increased oxidative stress, and increased activity of the RhoA/Rho-kinase signaling pathway.

Decreased erectile response in diabetic rats may be improved by adenovirus-mediated gene transfer of eNOS to the diabetic rat penis, causing an increase in cGMP formation (66). Moreover, the combination of eNOS gene therapy and sildenafil results in a synergistic increase in erectile response in diabetic rats (64), suggesting the impairment of NO-mediated responses. Increased activity and expression of arginase II in diabetic human cavernosal tissue may also decrease NO availability by competing with NOS for L-arginine. Inhibition of arginase has been shown to increase NO production in human diabetic penis (67). Another explanation for decreased eNOS activity in the diabetic penis may lie in reduced penile L-arginine content. Oral administration of L-arginine to diabetic rabbits increases endothelium-dependent relaxation of cavernosal tissue by improving NO biosynthesis (68).

Hyperglycemia is an important mediator of increased production of ROS leading to impaired endothelial function and structural impairment in the diabetic corpus cavernosum. The corpus cavernosum of diabetic rats and diabetic men with ED exhibits increased lipid peroxidation, upregulation of superoxide anion, and decreased antioxidants levels, suggestive of oxidative stress (69–71). Several studies have demonstrated improvement of endothelial function and erectile ability in diabetes with antioxidant therapy. Vitamin E and sodium selenate restore neuronal and endothelial function in the corpus cavernosum (72, 73). SOD and the metal chelator, trientine, improve relaxation of diabetic corpus cavernosum (74, 75). Overexpression of SOD *in vivo* by gene transfer to the diabetic rat penis reduces the increased superoxide anion levels and restores erectile function (69). These studies demonstrate that oxidative stress has a vital role in the development of ED associated with diabetes.

Increases in glucose promote the formation of advanced glycation end products (AGEs) formed between high levels of glucose, proteins, lipids, and nucleic acids. The action of AGEs is mostly mediated through cell surface receptors, such as the receptor for AGEs (RAGE), P60/OST48 protein (R-1), 80KH phosphoprotein (R-2) and galectin-3 (R-3), scavenger receptor II, lactoferrin-like polypeptide, and CD-36 (76). Some of the receptors are likely to contribute to clearance of AGEs, whereas others may mediate many of the adverse effects, such as quenching of NO, impairment of extracellular matrix and tissue remodeling, modification of circulating proteins, and receptor-mediated production of ROS. AGEs accumulate in diabetic cavernosal tissue, in particular, in endothelial and smooth muscle cells (77–80), and may be important in the pathogenesis of diabetes-induced ED. Inhibition of AGEs formation by aminoguanidine improves endothelium-dependent cavernosal smooth muscle relaxation *in vitro* (80) and erectile responses *in vivo* (78).

Hyperglycemia and increased oxidative stress in diabetes activate PKC by increasing *de novo* synthesis or accumulation of diacylglycerol. Activation of PKC, primarily the ß and δ isoforms, is associated with disordered NO production and abnormalities in blood flow and permeability (81). PKC may have multiple adverse effects on vascular function, including the activation of superoxide-producing enzymes (such as NAD(P)H oxidase), eNOS uncoupling, and inhibition of the activity and/or expression of the NO downstream target, soluble guanylyl cyclase (81). Corpus cavernosal vascular smooth muscle cells grown in a high glucose environment exhibit an upregulated PKCß2 isoform, with an associated increase in ROS and a decreased production of NO, which may be reversed with vitamin E (82). Inhibition of PKCßI and ßII isoforms attenuates endothelial dysfunction in the corpus cavernosum of diabetic mice (83). Thus, activation of PKC is associated with endothelial dysfunction in the diabetic penis, although the mechanisms are not fully understood.

Another mechanism that accounts for decreased NO formation in the diabetic penis involves decreased eNOS phosphorylation on Ser-1177 caused by O-linked N-acetylglucosamine (O-GlcNAc) modification of eNOS. This pathway is triggered by the activation of the hexosamine biosynthetic pathway, likely through hyperglycemia-induced mitochondrial overproduction of superoxide. O-linked GlcNAc is then attached to Ser and Thr residues of proteins involved in diverse aspects of cellular physiology, similarly to phosphorylation. This monosaccharide modification often competes in a ying-yang fashion with phosphorylation in the cell's regulatory pathways (84). In the diabetic rat penis, O-GlcNAc modification of eNOS decreases phosphorylation of the enzyme at Ser-1177 (but does not affect residues Thr-495, Ser-615, and Ser-633) both at baseline and in response to fluid shear stress stimuli and VEGF signaling. The diabetes-related deficit in basal eNOS activity seems to be caused by both O-GlcNAc modification of the enzyme and a loss of Akt-mediated phosphorylation of eNOS at Ser-1177 (85).

The specific mechanisms leading to diabetes-induced vascular complications, and ED in particular, are incompletely understood. A unifying concept has recently been proposed, asserting that hyperglycemia-induced increases in superoxide production by the mitochondrial electron transport chain results in accelerated AGE formation, activation of PKC, and increased hexosamine flux (86). However, this has not been investigated in diabetes-associated ED.

In addition to the effects of reduced NOS activity and NO production, the pathogenesis of diabetic ED is also

associated with increased erectile tissue contractility mediated *via* the RhoA/Rho-kinase pathway. Corpus cavernosal tissue obtained from alloxan-induced diabetic rabbits exhibits increased RhoA and Rho-kinase ß expression (87). The inhibition of RhoA expression in the penis of streptozotocin-induced diabetic rats by gene transfer of dominant negative RhoA increases eNOS protein content and restores erectile function (38), suggesting that upregulated Rho-kinase expression in the diabetic penis results from diminished endothelial NO production.

Hypercholesterolemia. Hypercholesterolemia and subsequent atherosclerosis is a significant risk factor for the development of vasculogenic ED. In men, the risk of ED increases 1.32-fold for every 1 n*M* increase in cholesterol (88). Despite numerous studies suggesting that increased plasma concentrations of LDL impair NO-dependent vasodilation, the molecular basis for the eNOS defect in atherosclerotic vasculature, and specifically the penile vascular bed, remains largely unknown.

Hypercholesterolemia in men and in cholesterol-fed animal models impairs endothelium-dependent relaxations (89–94) and endothelium-independent relaxations (89, 93) of the corpus cavernosum, decreases eNOS activity and NO bioavailability in the penis (94), decreases the cavernosal content of endothelial cells, alters the function of smooth muscle cells, and increases collagen content (89, 95–97). Erectile responses are also reduced in LDL receptor–null mice fed a high-cholesterol diet.[3] Impairment of the downstream mediator cGMP or subsequent signal transduction pathways has also been described in cavernosal tissue of hypercholesterolemic animals (98).

Impaired eNOS function and endothelial NO availability in the hypercholesterolemic vasculature has been mostly attributed to increased vascular superoxide production. Oxidative stress seems to contribute significantly to hypercholesterolemia-associated ED. Corpus cavernosal tissue of cholesterol-fed animals exhibits increased production of superoxide anion (90, 91, 98), possibly mediated by an increase in endogenous NAD(P)H oxidase expression or activity (98). Sildenafil normalizes cavernosal relaxation from hypercholesterolemic rabbits, in part, through suppression of NAD(P)H oxidase activity (98).

Oxidative modification of LDL (oxLDL), the major carrier of plasma cholesterol, plays a crucial role in hypercholesterolemia and atherosclerosis development. LDL can undergo oxidative modification by superoxide and peroxynitrite, and it accumulates in atherosclerotic plaques. OxLDL impairs eNOS function by several mechanisms, as demonstrated in endothelial cells from nonpenile tissue. It disrupts the organization of caveolae by removing caveolae cholesterol (99). Depletion of caveolae cholesterol results in the redistribution of caveolin-1 and eNOS to an intracellular membrane, where the enzyme is inaccessible to cofactors for its stimulation. OxLDL also increases the production of caveolin-1 and its association with eNOS (100), and decreases the association of eNOS with Hsp90 (99, 101), affecting the balance of NO and superoxide generation by eNOS and uncoupling eNOS activity. The effect of oxLDL on endothelial dysfunction has also been attributed to impaired generation of NO from eNOS because of dysregulation of its phosphorylation. In cultured endothelial cells exposed to atherogenic conditions, oxLDL has been reported to stimulate (101), attenuate (102), or have no effect (103) on phosphorylation of eNOS on Ser-1177, whereas it decreases phosphorylation on Thr-495 leading to uncoupling of eNOS and generation of superoxide (103). In human vascular endothelial cells, oxLDL stimulates oxidative stress *via* induction of NAD(P)H oxidase (104).

Besides its effect on endothelial function and its attenuation of endothelium-dependent vasodilation mediated by NO, oxLDL seems to directly affect smooth muscle cells independent of its effect on endothelial function. For example, in isolated rabbit aorta, oxLDL stimulates the RhoA pathway and potentiates Ang II-induced vasoconstriction (105).

Studies of the effect of oxLDL in ED are insufficient and inconclusive. Endothelium-dependent relaxation of rabbit corpus cavernosum strips *in vitro* was found to be unchanged (106) or decreased (91, 107) in response to oxLDL. Limited studies have also evaluated the effect of oxLDL on cavernosal smooth muscle contraction. OxLDL has been shown to produce a direct contractile effect (106) on corporal smooth muscles. No data are available on the role of the RhoA/Rho-kinase pathway in the penis in hypercholesterolemia-associated ED.

Hypertension. Essential or primary hypertension affects 20%–25% of all adults older than the age of 45–50 years. Approximately 30% of hypertension patients have ED. The degree of erectile impairment is directly correlated with the severity and duration of hypertension (108). Despite many epidemiologic studies showing the link between hypertension and ED, scientific studies are sparse, and the cellular and molecular mechanisms of impaired endothelial function in the penis and ED associated with hypertension are not fully understood.

Hypertension is characterized by increased peripheral resistance caused by alterations in the vascular endothelium, the surrounding vascular smooth muscle, and the associated extracellular matrix. Blood vessels in hypertension manifest decreased lumen diameter and thickening of the wall (109). Morphologic changes in the penis of hypertensive animals involve endothelial and smooth muscle damage, smooth muscle proliferation, increased collagen deposition, and thinning of the tunica albuginea (110–113). Functional impairment of the erectile tissue in hypertensive rats has been shown to result from decreased NO bioavailability, increased oxidative stress, and increased smooth muscle contraction. Both endothelium-dependent (112, 114), and

[3] Musicki B, Burnett AL. 2005. Unpublished data.

Table 1. Major Common Molecular Mechanisms of eNOS Dysfunction in Vasculogenic ED States.[a]

	Aging	Diabetes mellitus	Hypercholesterolemia	Hypertension
eNOS defect				
Substrate	+	+	–	–
Phosphorylation	+	+	–	–
Protein-protein interaction	–	–	–	–
ROS effect	–	–	–	–
eNOS regulatory proteins				
ROS effect	–	–	–	–
ROS				
Participation	+	+	+	+
Mechanism of production	–	–	+	–
RhoA/Rho-kinase				
Participation	+	+	–	+
Interaction with eNOS	–	+	–	–
ROS effect	–	–	–	–

[a]+, known to some extent; –, not known.

neurogenic NO- and carbon monoxide–dependent relaxation of the corpus cavernosum (115, 116) is decreased in hypertensive rats. Endothelium-independent relaxation of the erectile tissue is enhanced in spontaneously hypertensive rats, possibly as a compensatory mechanism resulting from defective endothelium-dependent relaxation (112, 114).

Angiotensin II is a potent vasoconstrictor implicated in the development and maintenance of hypertension. Inhibition of angiotensin II with angiotensin-converting enzyme inhibitors improves erectile function in hypertensive rats (117, 118). Within the vascular wall, angiotensin II stimulates the production of ROS through the activation of membrane-bound NAD(P)H oxidase (119). ROS generation is increased in blood vessels of patients with uncontrolled essential hypertension and different animal models of hypertension, whereas endothelial NO, SOD, vitamin E, and long-chain polyunsaturated fatty acids are decreased (120). Recent studies have shown that the corpus cavernosum of spontaneous hypertensive rats exhibits increased lipid peroxidation and decreased SOD levels (115), suggesting a role of oxidative stress in the impairment of penile erection associated with hypertension.

Increased peripheral vascular resistance in hypertension-associated ED is also associated with increased RhoA expression and Rho-kinase activity in the penis, whereas PDE5 inhibition and Rho-kinase inhibition synergistically potentiate the erectile response, implying impairment in NO/cGMP-mediated relaxation (116, 121). Increased cavernosal smooth muscle tone has also been attributed to a dysfunctional α-adrenergic contraction and increased cyclooxygenase-dependent constriction of the smooth muscle of the corpora cavernosa (114).

Complexity of eNOS Function in the Penis. Multiple post-translational levels of regulation of eNOS activity in the penis regulate eNOS-mediated responses under physiologic circumstances, and they provide various mechanisms whereby endothelial NO availability may be altered, leading to ED. It is apparent that certain common mechanisms underlie vascular pathophysiology in the penis associated with endothelial dysfunction of different origins. However, a specific mechanism affecting eNOS function may predominate in a specific vasculogenic ED state. For example, although Akt-dependent eNOS phosphorylation is impaired in both the aged and the diabetic rat penis, the underlying mechanisms are different: increased phosphorylation of a negative regulatory site Thr-495 on eNOS in the former (21), and increased O-GlcNAc modification of eNOS in the latter (85). Accordingly, the growth factor VEGF can rescue impaired erection in aged rats by stimulating downregulated eNOS phosphorylation at Ser-1177 (21), but it is ineffective in diabetic rats (85).

Conflicting information exists regarding eNOS expression in the penis associated with vasculogenic ED. For example, eNOS mRNA and protein expression in the aged penis was reportedly decreased (58) or increased (21, 48, 49), whereas the expression of eNOS in the diabetic penis is reportedly decreased (38) or unchanged (85, 122). Under physiologic conditions, an increase in eNOS protein is usually associated with an increase in the enzyme activity. However, the impairment of endothelial function is not necessarily caused by downregulation of eNOS protein. The elevated levels of eNOS protein in the penis may be adaptive and serve as a compensatory response to decreased endothelial NO production in an effort to maintain a certain level of erectile function, although the active form of eNOS is reduced. The activity of upregulated eNOS may be shifted toward increased production of superoxide. This may lead to uncoupling of upregulated eNOS, with subsequent potentiation of NO degradation and superoxide formation. This would result in a decrease in active eNOS, despite an increase in total eNOS expression. These findings argue against a simple notion that decreased eNOS gene expression accounts for endothelial dysfunction in different vascular beds, including the penis.

Conclusions

eNOS has an indispensable role in producing and maintaining a normal erectile response. It is regulated by multiple molecular mechanisms in the penis that may both positively and negatively affect its activity. Various mechanisms may disturb eNOS regulatory function and endothelial NO bioavailability, resulting in vasculogenic ED. As molecular mechanisms of normal erectile function and the pathways leading to vasculogenic ED associated with eNOS are becoming clearer, it seems that eNOS roles in the vascular pathophysiology of the penis are complicated and not always uniform. For example, eNOS phosphorylation in the penis is dysregulated with aging and diabetes, although by different mechanisms. However, increased oxidative stress in the penis seems to be a common component of vasculogenic ED, and activation of the RhoA/Rho-kinase contractile pathway is seen in several vasculogenic ED states. Table 1 summarizes unstudied or understudied scientific areas pertaining to dysregulated eNOS function in the penis associated with vasculogenic ED.

1. Ignarro LJ, Bush PA, Buga GM, Wood KS, Fukuto JM, Rajfer J. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem Biophys Res Commun 170:843–850, 1990.
2. Burnett AL, Lowenstein CJ, Bredt DS, Chang TS, Snyder SH. Nitric oxide: a physiologic mediator of penile erection. Science 257:401–403, 1992.
3. Busse R, Mulsch A. Calcium-dependent nitric oxide synthesis in endothelial cytosol is mediated by calmodulin. FEBS Lett 265:133–136, 1990.
4. 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 399:601–605, 1999.
5. 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 399:597–601, 1999.
6. Michell BJ, Griffiths JE, Mitchelhill KI, Rodriguez-Crespo I, Tiganis T, Bozinovski S, de Montellano PR, Kemp BE, Pearson RB. The Akt kinase signals directly to endothelial nitric oxide synthase. Curr Biol 9:845–848, 1999.
7. 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 278:14841–14849, 2003.
8. Boo YC, Sorescu G, Boyd N, Shiojimas I, Walsh K, Du J, Jo H. Shear stress stimulates phosphorylation of endothelial nitric oxide-synthase at Ser 1179 by Akt-independent mechanisms. Role of protein kinase A. J Biol Chem 277:3388–3396, 2002.
9. Sessa WC. eNOS at a glance. J Cell Sci 117:2427–2429, 2004.
10. Hurt, KJ, Musicki, B; Palese, MA, Crone, JK, Becker, RE, Moriarity, JL, Snyder, SH, Burnett, AL. Akt-dependent phosphorylation of endothelial nitric-oxide synthase mediates penile erection. Proc Natl Acad Sci U S A 99:4061–4066, 2002.
11. Musicki, B, Palese, MA, Crone, JK, Burnett, AL. Phosphorylated endothelial nitric oxide synthase mediates vascular endothelial growth factor-induced penile erection. Biol Reprod 70:282–289, 2004.
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 277:42344–42351, 2002.
13. Boo YC, Sorescu GP, Bauer PM, Fulton D, Kemp BE, Harrison DG, Sessa WC, Jo H. Endothelial NO synthase phosphorylated at SER635 produces NO without requiring intracellular calcium increase. Free Radic Biol Med 35:729–741, 2003.
14. 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 392:821–824, 1998.
15. Ou J, Fontana JT, Ou Z, Jones DW, Ackerman AW, Oldham KT, Yu J, Sessa WC, Pritchard KA Jr. Heat shock protein 90 and tyrosine kinase regulate eNOS NO* generation but not NO* bioactivity. Am J Physiol Heart Circ Physiol 286:H561–H569, 2004.
16. Pritchard KA Jr, Ackerman AW, Gross ER, Stepp DW, Shi Y Fontana YT, 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 276:17621–17624, 2001.
17. 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 276:16587–16591, 2001.
18. Michell BJ, Chen Z, 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 276:17625–17628, 2001.
19. 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 88:E68–E75, 2001.
20. Boo YC, Hwang J, Sykes M, Michell BJ, Kemp BE, Lum H, Jo H. Shear stress stimulates phosphorylation of eNOS at Ser 635 by a protein kinase A-dependent mechanism. Am J Physiol Heart Circ Physiol 283:H1819–H1828, 2002.
21. Musicki B, Kramer MF, Becker RE, Burnett AL. Age-related changes in phosphorylation of endothelial nitric oxide synthase in the rat penis. J Sex Med 2:347–357, 2005.
22. Sommer F, Klotz T, Steinritz D, Schmidt A, Addicks K, Engelmann U, Bloch W. MAP kinase 1/2 (Erk 1/2) and serine/threonine specific protein kinase Akt/PKB expression and activity in the human corpus cavernosum. Int J Impot Res 14:217–225, 2002.
23. Garcia-Cardena G, Oh P, Liu J, Schnitzer JE, Sessa WC. Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: implications for nitric oxide signaling. Proc Natl Acad Sci U S A 93: 6448–6453, 1996.
24. Feron, O, Saldana F, Michel JB, Michel T. The endothelial nitric-oxide synthase-caveolin regulatory cycle. J Biol Chem 273:3125–3128, 1998.
25. Michel JB, Feron O, Sacks D, Michel T. Reciprocal regulation of endothelial nitric-oxide synthase by Ca2+–calmodulin and caveolin. J Biol Chem 272:15583–15586, 1997.
26. Bucci M, Gratton JP, Rudic RD, Acevedo L, Roviezzo F, Cirino G, Sessa WC. In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. Nat Med 6: 1362–1367, 2000.
27. Nishida CR, Ortiz de Montellano PR. Autoinhibition of endothelial nitric-oxide synthase. Identification of an electron transfer control element. J Biol Chem 274:14692–14698, 1999.
28. Marrero MB, Venema VJ, Ju H, He H, Liang H, Caldwell RB, Venema RC. Endothelial nitric oxide synthase interactions with G-protein-coupled receptors. Biochem J 343:335–340, 1999.
29. Teixeira CE, Moreno RA, Ferreira U, Rodrigues Netto N Jr, Fregonesi A, Antunes E, De Nucci G. Pharmacological characterization of kinin-induced relaxation of human corpus cavernosum. Br J Urol 81:432–436, 1998.
30. Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T,

Tamakawa H, Yamagami K, Inui J, Maekawa M, Narumiya S. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 389:990–994, 1997.

31. Feng J, Ito M, Ichikawa K, Isaka N, Nishikawa M, Hartshorne DJ, Nakano T. Inhibitory phosphorylation site for Rho-associated kinase on smooth muscle myosin phosphatase. J Biol Chem 274:37385–37390, 1999.
32. Sauzeau V, Le Jeune H, Cario-Toumaniantz C, Smolenski A, Lohmann SM, Bertoglio J, Chardin P, Pacaud P, Loirand G. Cyclic GMP-dependent protein kinase signaling pathway inhibits RhoA-induced Ca^{2+} sensitization of contraction in vascular smooth muscle. J Biol Chem 275:21722–21729, 2000.
33. Ming XF, Viswambharan H, Barandier C, Ruffieux J, Kaibuchi K, Rusconi S, Yang Z. Rho GTPase/Rho kinase negatively regulates endothelial nitric oxide synthase phosphorylation through the inhibition of protein kinase B/Akt in human endothelial cells. Mol Cell Biol 22:8467–8477, 2002.
34. Chitaley K, Wingard CJ, Webb RC, Branam H, Stopper VS, Lewis RW, Mills TM. Antagonism of Rho-kinase stimulates rat penile erection via a nitric oxide-independent pathway. Nat Med 7:119–122, 2001.
35. Wang H, Eto M, Steers WD, Somlyo AP, Somlyo AV. RhoA-mediated Ca2+ sensitization in erectile function. J Biol Chem 277: 30614–30621, 2002.
36. Wingard CJ, Husain S, Williams J, James S. RhoA-Rho kinase mediates synergistic ET-1 and phenylephrine contraction of rat corpus cavernosum. Am J Physiol Regul Integr Comp Physiol 285:R1145–R1152, 2003.
37. Mills TM, Chitaley K, Lewis RW, Webb RC. Nitric oxide inhibits RhoA/Rho-kinase signaling to cause penile erection. Eur J Pharmacol 439:173–174, 2002.
38. Bivalacqua TJ, Champion HC, Usta MF, Cellek S, Chitaley K, Webb RC, Lewis RL, Mills TM, Hellstrom WJ, Kadowitz PJ. RhoA/Rho-kinase suppresses endothelial nitric oxide synthase in the penis: a mechanism for diabetes-associated erectile dysfunction. Proc Natl Acad Sci U S A 101:9121–9126, 2004.
39. Rees RW, Ralph DJ, Royle M, Moncada S, Cellek S. Y-27632, an inhibitor of Rho-kinase, antagonizes noradrenergic contractions in the rabbit and human penile corpus cavernosum. Br J Pharmacol 133: 455–458, 2001.
40. Teixeira CE, Ying Z, Webb RC. Pro-erectile effects of the Rho-kinase inhibitor (S)-(+)-2-methyl-1-[(4-methyl-5-isoquinolinyl)sulfonyl]homopiperazine (H-1152) in the rat penis. J Pharmacol Exp Ther 315: 155–162, 2005.
41. Chitaley K, Bivalacqua TJ, Champion HC, Usta MF, Hellstrom WJ, Mills TM, Webb RC. Adeno-associated viral gene transfer of dominant negative RhoA enhances erectile function in rats. Biochem Biophys Res Commun 298:427–432, 2002.
42. Touyz RM, Schiffrin EL. Reactive oxygen species in vascular biology: implications in hypertension. Histochem Cell Biol 2004 122: 339–352, 2004.
43. Schulz E, Anter E, Keaney JF Jr. Oxidative stress, antioxidants, and endothelial function. Curr Med Chem 11:1093–1104, 2004.
44. Bivalacqua TJ, Armstrong JS, Biggerstaff J, Abdel-Mageed AB, Kadowitz PJ, Hellstrom WJ, Champion HC. Gene transfer of extracellular SOD to the penis reduces O2-* and improves erectile function in aged rats. Am J Physiol Heart Circ Physiol 284:H1408–H1421, 2003.
45. Jin L, Ying Z, Webb RC. Activation of Rho/Rho kinase signaling pathway by reactive oxygen species in rat aorta. Am J Physiol Heart Circ Physiol 287:H1495–H1500, 2004.
46. Meuleman EJ. Prevalence of erectile dysfunction: need for treatment? Int J Impot Res 14 (Suppl 1):S22–S28, 2002.
47. Garban H, Vernet D, Freedman A, Rajfer J, Gonzalez-Cadavid N. Effect of aging on nitric oxide-mediated penile erection in rats. Am J Physiol 268:H467–H475, 1995.
48. Haas C, Seftel AD, Razmjouei K, Ganz MB, Hampel N, Ferguson K. Erectile dysfunction in aging: upregulation of endothelial nitric oxide synthase. Urology 51:516–522, 1998.
49. Bakircioglu ME, Sievert KD, Nunes L, Lau A, Lin CS, Lue TF. Decreased trabecular smooth muscle and caveolin-1 expression in the penile tissue of aged rats. J Urol 166:734–738, 2001.
50. Gonzalez-Cadavid NF, Rajfer J. Molecular pathophysiology and gene therapy of aging-related erectile dysfunction. Exp Gerontol 39:1705–1712, 2004.
51. Seftel AD. Erectile dysfunction in the elderly: epidemiology, etiology and approaches to treatment. J Urol 169:1999–2007, 2003.
52. Champion HC, Bivalacqua TJ, Hyman AL, Ignarro LJ, Hellstrom WJ, Kadowitz PJ. Gene transfer of endothelial nitric oxide synthase to the penis augments erectile responses in the aged rat. Proc Natl Acad Sci U S A 96:11648–11652, 1999.
53. Lin CS, Liu X, Chow S, Lue TF. Cyclic AMP and cyclic GMP activate protein kinase G in cavernosal smooth muscle cells: old age is a negative factor. BJU Int 89:576–582, 2002.
54. Musicki B, Champion HC, Becker RE, Liu T, Kramer MF, Burnett AL. Erection capability is potentiated by chronic sildenafil treatment: role of blood flow-induced endothelial nitric oxide synthase phosphorylation. Mol Pharmacol 68:226–232, 2005.
55. Sakai Y, Masuda H, Kihara K, Kurosaki E, Yamauchi Y, Azuma H. Involvement of increased arginase activity in impaired cavernous relaxation with aging in the rabbit. J Urol 172:369–373, 2004.
56. Moody JA, Vernet D, Laidlaw S, Rajfer J, Gonzalez-Cadavid N. Effect of long-term oral administration of L-arginine on the rat erectile response. J Urol 158:942–947, 1997.
57. Ferrini M, Magee TR, Vernet D, RajferJ, Gonzalez-Cadavid NF. Aging-related expression of inducible nitric oxide synthase and markers of tissue damage in the rat penis. Biol Reprod 64:974–982, 2001.
58. Rajasekaran, M, Kasyan, A, Jain, A, Kim, SW, Monga, M. Altered growth factor expression in the aging penis: the Brown-Norway rat model. J Androl 23:393–399, 2002.
59. Rajasekaran M, White S, Baquir A, Wilkes N. Rho-kinase inhibition improves erectile function in aging male Brown-Norway rats. J Androl 26:182–188, 2005.
60. Hakim LS, Goldstein I. Diabetic sexual dysfunction. Endocrinol Metab Clin North Am 25:379–400, 1996.
61. Saenz de Tejada I, Goldstein I, Azadzoi K, Krane RJ, Cohen RA. Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. N Engl J Med 320: 1025–1030, 1989.
62. Cellek S, Foxwell NA, Moncada S. Two phases of nitrergic neuropathy in streptozotocin-induced diabetic rats. Diabetes 52: 2353–2362, 2003.
63. Podlasek CA, Zelner DJ, Bervig TR, Gonzalez CM, McKenna KE, McVary KT. Characterization and localization of nitric oxide synthase isoforms in the BB/WOR diabetic rat. J Urol 166:746–755, 2001.
64. Bivalacqua TJ, Usta MF, Champion HC, Leungwattanakij S, Dabisch PA, McNamara DB, Kadowitz PJ, Hellstrom WJ. Effect of combination endothelial nitric oxide synthase gene therapy and sildenafil on erectile function in diabetic rats. Int J Impot Res 16:21–29, 2004.
65. Chang S, Hypolite JA, Velez M, Changolkar A, Wein AJ, Chacko S, DiSanto ME. Downregulation of cGMP-dependent protein kinase-1 activity in the corpus cavernosum smooth muscle of diabetic rabbits. Am J Physiol Regul Integr Comp Physiol 287:R950–960, 2004.
66. Bivalacqua TJ, Usta MF, Champion HC, Adams D, Namara DB, Abdel-Mageed AB, Kadowitz PJ, Hellstrom WJ. Gene transfer of endothelial nitric oxide synthase partially restores nitric oxide

synthesis and erectile function in streptozotocin diabetic rats. J Urol 169:1911–1917, 2003.
67. Bivalacqua TJ, Hellstrom WJG, Kadowitz PJ, Champion HC. Increased expression of arginase II in human diabetic corpus cavernosum: in diabetic-associated erectile dysfunction. Biochem Biophys Res Commun 283:923–927, 2001.
68. Yildirim S, Ayan S, Sarioglu Y, Gultekin Y, Butuner C. The effects of long-term oral administration of L-arginine on the erectile response of rabbits with alloxan-induced diabetes. BJU Int 83:679–685, 1999.
69. Bivalacqua TJ, Usta MF, Kendirci M, Pradhan L, Alvarez X, Champion HC, Kadowitz PJ, Hellstrom WJG. Superoxide anion production in the rat penis impairs erectile function in diabetes: influence of in vivo extracellular superoxide dismutase gene therapy. J Sex Med 2:187–198, 2005.
70. Ryu JK, Kim DJ, Lee T, Kang YS, Yoon SM, Suh JK. The role of free radical in the pathogenesis of impotence in streptozotocin-induced diabetic rats. Yonsei Med J 44:236–241, 2003.
71. Tuncayengin A, Biri H, Onaran M, Sen I, Tuncayengin O, Polat F, Erbas D, Bozkirli I. Cavernosal tissue nitrite, nitrate, malondialdehyde and glutathione levels in diabetic and non-diabetic erectile dysfunction. Int J Androl 26:250–254, 2003.
72. De Young L, Yu D, Bateman RM, Brock GB. Oxidative stress and antioxidant therapy: their impact in diabetes-associated erectile dysfunction. J Androl 25:830–836, 2004.
73. Gocmen C, Secilmis A, Kumcu EK, Ertug PU, Onder S, Dikmen A, Baysal F. Effects of vitamin E and sodium selenate on neurogenic and endothelial relaxation of corpus cavernosum in the diabetic mouse. Eur J Pharmacol 398:93–98, 2000.
74. Khan MA, Thompson CS, Jeremy JY, Mumtaz FH, Mikhailidis P, Morgan RJ. The effect of superoxide dismutase on nitric oxide-mediated and electrical field-stimulated diabetic rabbit cavernosal smooth muscle relaxation. BJU Int 87:98–103, 2001.
75. Keegan A, Cotter MA, Cameron NE. Effects of chelator treatment on aorta and corpus cavernosum from diabetic rats. Free Radic Biol Med 27:536–543, 1999.
76. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovasc Res 63:582–592, 2004.
77. Seftel AD, Vaziri ND, Ni Z, Razmjouei K, Fogarty J, Hampel N, Polak J, Wang RZ, Ferguson K, Block C, Haas C. Advanced glycation end products in human penis: elevation in diabetic tissue, site of deposition, and possible effect through iNOS or eNOS. Urology 50: 1016–1026, 1997.
78. Usta MF, Bivalacqua TJ, Yang DY, Ramanitharan A, Sell DR, Viswanathan A, Monnier VM, Hellstrom WJ. The protective effect of aminoguanidine on erectile function in streptozotocin diabetic rats. J Urol 170:1437–1442, 2003.
79. Cartledge JJ, Eardley I, Morrison JF. Advanced glycation end-products are responsible for the impairment of corpus cavernosal smooth muscle relaxation seen in diabetes. BJU Int 87:402–407, 2001.
80. Cellek S, Qu W, Schmidt AM, Moncada S. Synergistic action of advanced glycation end products and endogenous nitric oxide leads to neuronal apoptosis in vitro: a new insight into selective nitrergic neuropathy in diabetes. Diabetologia 47:331–339, 2004.
81. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 414:813–820, 2001.
82. Ganz MB, Seftel A. Glucose-induced changes in protein kinase C and nitric oxide are prevented by vitamin E. Am J Physiol Endocrinol Metab 278:E146–E152, 2000.
83. Nangle MR, Cotter MA, Cameron NE. Protein kinase C beta inhibition and aorta and corpus cavernosum function in streptozotocin-diabetic mice. Eur J Pharmacol 475:99–106, 2003.
84. Wells L, Vosseller K, Hart GW. Glycosylation of nucleocytoplasmic proteins: signal transduction and O-GlcNAc. Science 291:2376–2378, 2001.
85. Musicki B, Kramer MF, Becker RE, Burnett AL. Inactivation of phosphorylated endothelial nitric oxide synthase (Ser-1177) by O-GlcNAc in diabetes-associated erectile dysfunction. Proc Natl Acad Sci U S A 102:11870–11875, 2005.
86. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 54:1615–1625, 2005.
87. Chang S, Hypolite JA, Changolkar A, Wein AJ, Chacko S, DiSanto ME. Increased contractility of diabetic rabbit corpora smooth muscle in response to endothelin is mediated via Rho-kinase beta. Int J Impot Res 15:53–62, 2003.
88. Wei M, Macera CA, Davis DR, Hornung CA, Nankin HR, Blair SN. Total cholesterol and high density lipoprotein cholesterol as important predictors of erectile dysfunction. Am J Epidemiol 140:930–937, 1994.
89. Kim JH, Klyachkin ML, Svendsen E, Davies MG, Hagen PO, Carson CC 3rd. Experimental hypercholesterolemia in rabbits induces cavernosal atherosclerosis with endothelial and smooth muscle cell dysfunction. J Urol 151:198–205, 1994.
90. Kim SC, Kim IK, Seo KK, Baek KJ, Lee MY. Involvement of superoxide radical in the impaired endothelium-dependent relaxation of cavernous smooth muscle in hypercholesterolemic rabbits. Urol Res 25:341–346, 1997.
91. Kim SC. Hyperlipidemia and erectile dysfunction. Asian J Androl 2: 161–166, 2000.
92. Azadzoi KM, Saenz de Tejada I. Hypercholesterolemia impairs endothelium-dependent relaxation of rabbit corpus cavernosum smooth muscle. J Urol 146:238–240, 1991.
93. Behr-Roussel D, Bernabe J, Compagnie S, Rupin A, Verbeuren TJ, Alexandre L, Giuliano F. Distinct mechanisms implicated in atherosclerosis-induced erectile dysfunction in rabbits. Atherosclerosis 162:355–362, 2002.
94. Seo KK, Yun HY, Kim H, Kim SC. Involvement of endothelial nitric oxide synthase in the impaired endothelium-dependent relaxation of cavernous smooth muscle in hypercholesterolemic rabbit. J Androl 20: 298–306, 1999.
95. Yesilli C, Yaman O, Anafarta K. Effect of experimental hypercholesterolemia on cavernosal structures. Urology 57:1184–1188, 2001.
96. Gholami SS, Rogers R, Chang J, Ho HC, Grazziottin T, Lin CS, Lue TF. The effect of vascular endothelial growth factor and adeno-associated virus mediated brain derived neurotrophic factor on neurogenic and vasculogenic erectile dysfunction induced by hyperlipidemia. J Urol 169:1577–1581, 2003.
97. Nehra A, Azadzoi KM, Moreland RB, Pabby A, Siroky MB, Krane RJ, Robert J, Goldstein I, Udelson D. Cavernosal expandability is an erectile tissue mechanical property which predicts trabecular histology in an animal model of vasculogenic erectile dysfunction. J Urol 159: 2229–2236, 1998.
98. Shukla N, Jones R, Persad R, Angelini GD, Jeremy JY. Effect of sildenafil citrate and a nitric oxide donating sildenafil derivative, NCX 911, on cavernosal relaxation and superoxide formation in hypercholesterolaemic rabbits. Eur J Pharmacol 517:224–231, 2005.
99. Blair A, Shaul PW, Yuhanna IS, Conrad PA, Smart EJ. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmalemmal caveolae and impairs eNOS activation. J Biol Chem 274:32512–32519, 1999.
100. Feron O, Dessy C, Moniotte S, Desager JP, Balligand JL. Hypercholesterolemia decreases nitric oxide production by promoting the interaction of caveolin and endothelial nitric oxide synthase. J Clin Invest 103:897–905, 1999.
101. Pritchard KA, Ackerman AW, Ou J, Curtis M, Smalley DM, Fontana JT, Stemerman MB, Sessa WC. Native low-density lipoprotein induces endothelial nitric oxide synthase dysfunction: role of heat shock protein 90 and caveolin-1. Free Radic Biol Med 33:52–62, 2002.

102. Chavakis E, Dernbach E, Hermann C, Mondorf UF, Zeiher AM, Dimmeler S. Oxidized LDL inhibits vascular endothelial growth factor-induced endothelial cell migration by an inhibitory effect on the Akt/endothelial nitric oxide synthase pathway. Circulation 24:2102–2107, 2001.
103. Fleming I, Mohamed A, Galle J, Turchanowa L, Brandes RP, Fisslthaler B, Busse R. Oxidized low-density lipoprotein increases superoxide production by endothelial nitric oxide synthase by inhibiting PKCalpha. Cardiovasc Res 1:897–906, 2005.
104. Rueckschloss U, Galle J, Holtz J, Zerkowski H-R, Morawietz H. Induction of NAD(P)H oxidase by oxidized low-density lipoprotein in human endothelial cells: antiatherosclerotic potential of HMG-CoA reductase inhibitor therapy. Circulation 104:1767–1772, 2001.
105. Galle J, Mameghani A, Bolz SS, Gambaryan S, Gorg M, Quaschning T, Raff U, Barth H, Seibold S, Wanner C, Pohl U. Oxidized LDL and its compound lysophosphatidylcholine potentiate Ang II-induced vasoconstriction by stimulation of RhoA. J Am Soc Nephrol 14: 1471–1479, 2003.
106. Ahn TY, Gomez-Coronado D, Martinez V, Cuevas P, Goldstein I, Saenz de Tejada I. Enhanced contractility of rabbit corpus cavernosum smooth muscle by oxidized low density lipoproteins. Int J Impot Res 11:9–14, 1999.
107. Kim SC, Seo KK, Kim HW, Lee MY. The effects of isolated lipoproteins and triglyceride, combined oxidized low density lipoprotein (LDL) plus triglyceride, and combined oxidized LDL plus high density lipoprotein on the contractile and relaxation response of rabbit cavernous smooth muscle. Int J Androl 23(Suppl 2):26–29, 2000.
108. Burchardt M, Burchardt T, Baer L, Kiss AJ, Pawar RV, Shabsigh A, de la Taille, Hayek OR, Shabsigh R. Hypertension is associated with severe erectile dysfunction. J Urol 164:1188–1191, 2000.
109. Mulvany MJ. Vascular remodelling of resistance vessels: can we define this? Cardiovasc Res 41:9–13, 1999.
110. Toblli JE, Stella I, Inerra F, Ferder L, Zeller F, Mazza ON. Morphological changes in cavernous tissue in spontaneously hypertensive rats. Am J Hypertension 13:686–692, 2000.
111. Jiang R, Chen JH, Jin J, Shen W, Li QM. Ultrastructural comparison of penile cavernous tissue between hypertensive and normotensive rats. Int J Impot Res (Epub ahead of print), 2005.
112. Behr-Roussel D, Gorny D, Mevel K, Compagnie S, Kern P, Sivan V, Bernabe J, Bedigian MP, Alexandre L, Giuliano F. Erectile dysfunction: an early marker for hypertension? A longitudinal study in spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol 288:R276–R283, 2005.
113. Okabe H, Hale TM, Kumon H, Heaton JP, Adams MA. The penis is not protected—in hypertension there are vascular changes in the penis which are similar to those in other vascular beds. Int J Impot Res 11: 133–140, 1999.
114. Behr-Roussel D, Chamiot-Clerc P, Bernabe J, Mevel K, Alexandre L, Safar ME, Guiliano F. Erectile dysfunction in spontaneously hypertensive rats: pathophysiological mechanisms. Am J Physiol Regul Integr Comp Physiol 284:R682–R688, 2003.
115. Ushiyama M, Morita T, Kuramochi T, Yagi S, Katayama S. Erectile dysfunction in hypertensive rats results from impairment of the relaxation evoked by neurogenic carbon monoxide and nitric oxide. Hypertens Res 27:253–261, 2004.
116. Wilkes N, White S, Stein P, Bernie J, Rajasekaran M. Phosphodiesterase-5 inhibition synergizes rho-kinase antagonism and enhances erectile response in male hypertensive rats. Int J Impot Res 16:187–194, 2004.
117. Dorrance AM, Lewis RW, Mills TM. Captopril reverses erectile dysfunction in male stroke prone spontaneously hypertensive rats. Int J Impot Res 14:494–497, 2002.
118. Hale TM, Okabe H, Bushfield TL, Heaton JP, Adams MA. Recovery of erectile function after brief aggressive antihypertensive therapy. J Urol 168:348–354, 2002.
119. Touyz RM. Intracellular mechanisms involved in vascular remodelling of resistance arteries in hypertension: role of angiotensin II. Exp Physiol 90:449–455, 2005.
120. Das UN. Long-chain polyunsaturated fatty acids interact with nitric oxide, superoxide anion, and transforming growth factor-ß to prevent human essential hypertension. Eur J Clin Nutr 58:195–203, 2004.
121. Chitaley K, Webb RC, Dorrance AM, Mills TM. Decreased penile erection in DOCA-salt and stroke prone-spontaneously hypertensive rats. Int J Impot Res 13(Suppl 5):S16–S20, 2001.
122. Escrig A, Marin R, Abreu P, Gonzalez-Mora JL, Mas M. Changes in mating behavior, erectile function, and nitric oxide levels in penile corpora cavernosa in streptozotocin-diabetic rats. Biol Reprod 66: 185–189, 2002.