INTRODUCTION

Penile erection is a complex neurovascular process involving increased arterial inflow into the penis, restricted venous outflow from the penis, and relaxation of the corpus cavernosal smooth muscle. The process relies on coordination of the nervous system (including the hypotalamus, spinal cord, and peripheral nerves in the penis), and the vascular and sinusoidal endothelium (endothelium of arteries, cavernosal cisternae) of the penis. NO synthase (eNOS, NOS3) and neuronal NO synthase (nNOS, NOS1) isoforms are tightly regulated and produce physiologically relevant levels of NO in the penis required for the erectile response. Inducible NOS (iNOS; NOS2) is independent of calcium and calmodulin and requires new protein synthesis. nNOS is mostly localized to nitrergic nerve terminals of penile autonomic nerves (pelvic plexus, cavernous and dorsal nerves), and eNOS is mostly localized to the vascular and sinusoidal endothelium (endothelium of the arteries and cavernosal cisternae) of the penis. NO synthesized by nNOS and eNOS diffuses to the underlying corporal smooth muscle cells where it activates the soluble form of guanylate cyclase thus elevating intracellular levels of cGMP (4). cGMP activates cGMP-dependent protein kinase G-1 (PKG), which in turn phosphorylates gap junctions and calcium and potassium channels, inhibiting calcium mobilization from intracellular stores and calcium influx, and antagonizing the RhoA/Rho-kinase pathway (4). This biochemical process results in relaxation of vascular smooth muscle cells in the arteries, arterioles, and sinusoids of the corpora cavernosa, and penile erection (Figure 1).
Degradation of cGMP in the penis to an inactive 5'-GMP, which terminates NO signaling pathway and returns the penis to the flaccid state, is catalyzed primarily by phosphodiesterase (PDE)5 (5-7).

1.1. Constitutive nitric oxide synthases

NOS isoenzymes are homodimeric oxidoreductases. Each monomer has two functionally different domains, an N-terminal oxygenase domain which binds heme, a cofactor 5,6,7,8-tetrahydrirobioprotein (BH4), molecular oxygen, and the substrate L-arginine, and a C-terminal reductase domain, which binds nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). The two domains are linked by a short sequence that binds calmodulin. NOSs catalyze electron transfer from the C-terminally bound NADPH via the flavins to the heme on the N terminus of the other monomer. Because electrons flow from the reductase domain of one monomer to the oxygenase domain of another monomer, enzyme dimerization is required for full enzymatic activity. Calcium/calmodulin activation causes conformational changes in the enzyme and increases the rate of electron flow. At the heme, electrons reduce and activate oxygen, resulting in oxidation of L-arginine to NO and L-citrulline (8).

Both eNOS and nNOS are regulated transcriptionally and posttranslationally. Transcriptional regulation modulates long term NO production in the penis. Due to the necessity for rapid production of NO for physiologic penile erection, posttranslational regulation appears to be more relevant. Importantly, posttranslational regulation of eNOS, and possibly nNOS, contributes to vascular homeostasis in the penis.

Given the impact of endothelial- and neuronal-derived NO in penile biology, a great deal of research over the past decade has focused on the role of NO synthesis from the endothelium and nitrergic nerve terminal in erection physiology and disease states associated with ED. Common factors contributing to ED include reduced activity of nNOS and eNOS and decreased NO bioavailability.

1.1.1. nNOS

Nitrergic neurotransmission refers to neuronal NO signaling in penile erection. The nitrergic activation of penile erection depends on discrete brain regions such as the medial preoptic area (MPOA) and the paraventricular nucleus (PVN) within the hypothalamus where the erectile stimuli originate, the L6-S1 region of the spinal cord, and peripheral nerves of the corpora cavernosa (9).

nNOS activity is regulated at the transcriptional level and by multi-site phosphorylation, protein-protein interactions, and subcellular localization. Psychogenic and reflexogenic (tactile) stimuli initiate penile erection through the activation of nNOS. This is achieved mainly by depolarization-induced calcium entry and calcium binding to calmodulin by means of calcium flux through the N-methyl-D-aspartate receptor (NMDAR). NMDAR is coupled to nNOS by the binding protein post synaptic density-95 (PSD95) through interaction with the NOS PDZ domain. The association of nNOS with PSD95 is necessary for NOS activation by the NMDAR (10). Transient activation of nNOS by the NMDAR appears to involve phosphorylation of nNOS at activating and inhibitory sites Ser-1412 and Ser-847, respectively. It has been postulated that activation of NMDAR leads to phosphorylation at Ser-1412 on eNOS and enzyme activation. Phosphorylation at Ser-1412 also serves to expose Ser-847 in the auto-inhibitory domain of nNOS which is phosphorylated by calcium/calmodulin dependent kinase II resulting in enzyme deactivation (11). Whether similar mechanisms operate in the penis, however, is not known.

Both the NMDAR and inhibitors of nNOS activity, such as protein inhibitor of NOS (PIN), and the carboxyterminal PDZ ligand of nNOS (CAPON), are expressed in pelvic ganglia and penile nerves (PIN, NMDAR, CAPON) and in the hypothalamic and spinal cord regions involved in penile erection (PIN; 10).

PnNOS, the nNOS variant expressed in the penis and prostate, exists as two isoforms, a full-length alpha and a beta isoform, which lacks the N-terminal PDZ domain, important for protein-protein interactions. Both alternatively spliced forms of nNOS mediate penile erection (10). Derangement of nNOS expression and nNOS activity, including its interaction with its modulators, may compromise nitrergic control of erection.

Figure 1. NO-mediated penile erection. NO is synthesized from its precursor L-arginine by nNOS and eNOS, primarily localized in autonomic nerve endings and endothelial cells of the penis, respectively. nNOS is activated in response to psychogenic and reflexogenic neuronal impulses. eNOS is activated in response to agonists such as acetylcholine or Bradykinin, but the most important physiologic agonist for eNOS is shear stress, a pressure exerted on endothelial cells by blood flow in the vessel at a constant flow rate. NO diffuses to adjacent smooth muscle cells where it activates soluble guanylyl cyclase (sGC) and increases the production of 3',5'-cyclic guanosine monophosphate (cGMP) from 5'-guanosine triphosphate (GTP). Subsequent activation of cGMP-specific protein kinase I (PKG) reduces contractile activity and promotes relaxation of smooth muscle cells and erection. Degradation of cGMP in the penis to inactive 5'-GMP, which terminates NO signaling and returns the penis to the flaccid state, is catalyzed primarily by type 3 phosphodiesterase (PDE5).

Figure 2. A model depicting initiation of erection by nNOS and its maintenance by eNOS. In response to sexual stimuli, depolarization-induced calcium entry and calcium/calmodulin activation of nNOS initiates erection through a relatively short-lived burst of NO. Vasorelaxation causes increased blood flow and physical expansion of penile vasuctalure and sinusoidal spaces. Calcium-"independent" eNOS phosphorylation on Ser-1177 and activation by the resulting shear stress provides a sustained increase in NO, accounting for the achievement and maintenance of full erection.

Initiation of erection by nNOS

Maintenance of erection by eNOS

P-eNOS (Ser-1177)

nNOS → NO → Shear stress

Continued relaxation

Shear stress

NO

P-eNOS (Ser-1177)
1.1.2. Other neurotransmitters

A host of neurotransmitters besides NO are involved in penile erection. At the levels of brain and spinal cord they include facilitatory (such as dopamine, acetylcholine, oxytocin and adrenocorticotropic/c-t-melanocyte-stimulating hormone), or inhibitory (such as gamma aminobutyric acid [GABA], serotonin, and opioid peptides) neurotransmitters of penile erection. (4)

1.1.3. eNOS

In normal endothelial function, endothelial NO has vasodilator 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.

eNOS is regulated transcriptionally and posttranslationally. The latter mechanisms involve calcium/calmodulin binding, fatty acid modification, alterations in intracellular translocation, substrate and cofactor availability, dimerization of the enzyme subunits, binding to other proteins, such as caveolin-1 and heat shock protein (Hsp) 90, and multisite phosphorylation. Interaction of eNOS with Hsp90 positively regulates the enzyme’s activity, whereas interaction of eNOS with caveolin-1 negatively regulates the enzyme’s activity. Activation of eNOS with calcium/calmodulin is essential to the first step of the catalytic process of NO production. Phosphorylation of eNOS at Ser-1177 is a key post-translational modification, which reduces calmodulin dissociation from eNOS and facilitates electron transfer, thus ensuring a constitutive production of NO at low intracellular calcium levels (12).

Studies performed in the past several years have established an indispensable role of eNOS in penile erection (6, 7, 13-19). The classical concept of nNOS and eNOS activation by calcium/calmodulin in the penis accounts for rapid and transient production of NO. According to new concepts, nNOS and eNOS, respectively, mediate the initiation and maintenance of penile erection (Figure 2). In response to sexual stimuli depolarization-induced calcium entry and calcium/calmodulin activation of nNOS initiates vasorelaxation and penile erection. The activation of eNOS in response to agonists such as acetylcholine or bradykinin is also induced by increases in intracellular calcium. Vasorelaxation causes increased blood flow and physical expansion of penile vasculature and sinusoidal spaces. The resulting shear force on the endothelium of these structures activates eNOS phosphorylation at Ser-1177, resulting in reduced calcium dependence and sustained release of endothelial NO, accounting for the achievement and maintenance of full erection (13). In addition, the maintenance of eNOS in its dimeric form and limited eNOS interaction with its negative regulator caveolin-1 are prerequisites for physiologic penile erection (16). Derangements in any of the molecular pathway which regulate eNOS may induce vasogenic ED.

1.1.4. NO-independent endothelium-derived relaxing factors

Endothelial cells release other vasodilators besides NO, including prostacyclin and endothelium-derived hyperpolarizing factor (EDHF). Prostacyclin, synthesized by both the endothelium and smooth muscle, is involved in cAMP-mediated smooth muscle relaxation. Endothelium-dependent vasodilation in smaller arteries and arterioles is mostly attributed to non-NO/nonprostacyclin-induced hyperpolarization mediated by EDHF. There is considerable controversy regarding the identity and the mechanism of action of EDHF. It is thought that the hyperpolarization of smooth muscle cell membrane by EDHF predominantly involves activation of calcium-dependent potassium channels (20). Decreased NO-independent vasorelaxation in the penis may lead to ED.

1.2. Smooth muscle mediators

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 (vasoconstrictive) mechanisms, which operate physiologically in the penis. Vasoconstriction is evoked by norepinephrine through α-adrenergic receptors, endothelins, angiotensin 2, and thromboxane A2. Agonist-induced activation of G-protein coupled receptors increases influx of extracellular calcium and increases phospholipase C activity. Phospholipase C cleaves membrane-bound phosphatidylinositol into inositol trisphosphate and diacylglycerol, resulting in an elevation of intracellular calcium levels. During this phase, calcium/calmodulin activates myosin light chain kinase, leading to increased phosphorylation of myosin light chain, actin/myosin assembly, and smooth muscle contraction (20, 21).

![Figure 3. RhoA/Rho-kinase pathway in penile smooth muscle contraction](image-url)
1.2.1. RhoA/Rho-kinase pathway

While smooth muscle contraction is regulated primarily by cytosolic calcium, once the calcium levels return to basal levels, the calcium-independent increase in vascular smooth muscle tone, known as calcium-sensitization, takes over. This pathway is largely mediated by activation of the small GT-Pase, RhoA, and its downstream effector, Rho-kinase. 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 thereby inhibits regulatory myosin phosphatase target subunit 1 of myosin light chain phosphatase at Thr-696 and inhibits its activity. Inhibition of myosin light chain phosphatase increases myosin light chain phosphorylation, which promotes the actin-myosin cross bridge cycling rate, promoting smooth muscle contraction at low calcium levels (3, 22).

An inverse functional relationship exists between the NO/cGMP/PKG 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. The RhoA/Rho-kinase pathway downregulates eNOS gene expression through post-transcriptional mRNA destabilization. In addition, in human endothelial cells, the RhoA/Rho-kinase pathway inhibits eNOS phosphorylation at its positive regulatory site Ser-1177, and stimulates eNOS phosphorylation at its negative regulatory site Thr-495, suppressing NO production (18). Figure 3 schematically depicts regulation of the contractile pathway in smooth muscle and its interaction with the NO relaxant pathway.

RhoA/Rho-kinase pathway plays an important role in maintaining the penis in the flaccid state. During erection, this pathway is inhibited, most likely by NO. Conversely, RhoA/Rho-kinase suppresses eNOS gene expression and enzyme activity in the penis (3). Upregulated function or activity of the RhoA/Rho-kinase pathway may increase smooth muscle contractility and result in ED.

1.3. Reactive oxygen species (ROS)

Superoxide anion is produced in a variety of cells, including vascular smooth muscle cells and endothelial cells. Many other ROS are formed secondary to reactions involving superoxide. Potential vascular sources of superoxide include nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase, uncoupled eNOS, xanthine oxidase, peroxisomal oxidases, cyclooxygenases and mitochondrial electron transport (23). ROS sources may interact with each other, and activation of one may augment the activity of others. ROS may affect NO activity, endothelial and neuronal NO availability, smooth muscle cell integrity, and RhoA/Rho-kinase pathway function. Superoxide may inactivate NO and decrease its bioavailability, or it may directly affect NO through transcriptional mechanisms or post-translationally by preventing NO denitrosylation via redox-sensitive cell-signaling pathways. Moreover, the reaction of superoxide and NO results in the formation of reactive nitrogen species such as the highly toxic molecule peroxynitrite. Peroxynitrite may cause oxidative damage to DNA, proteins and lipids, NO uncoupling, promote release of vasoconstrictors, increase apoptosis, and cause tissue injury and inflammation. ROS scavengers and enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, eliminate ROS within vasculature (23).

Endothelial and smooth muscle cells of the penis contain cytosolic copper/zinc and extracellular isoforms of SOD (18, 24). The imbalance between the production and elimination of ROS, a condition known as oxidative stress, has an important role in the development of ED.

1.4. Growth factors, cytokines, and neurotrophic factors

Growth factors act as paracrine and autocrine regulators in the vasculature. Vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF) and its binding proteins, basic fibroblast growth factor (bFGF), and transforming growth factor (TGF)β1 have been characterized in the penis. VEGF is an angiogenic factor which directly activates eNOS and regulates apoptosis. IGF-1 has been implicated in the regulation of vascular smooth muscle proliferation and migration, and plays a role in the control of cell growth, cell survival, and mitogenesis. The cytokine TGFβ1 promotes the synthesis of collagen and inhibits growth of vascular smooth muscle cells (25).

Many paracrine and autocrine factors can modulate the erectile response by affecting neuronal development and neuron regeneration. They include classic neurotrophins (such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), and neurotrophin 4 (NT4)), growth factors (fibroblast growth factor, VEGF, IGF-1 and IGFBP3), immunophilin ligands and atypical neurotrophic factors (such as growth hormones, the glial cell derived neuritun, the morphogenetic Sonic hedgehog protein, and erythropoietin). Impaired growth factor, cytokine, and neurotrophin production and signaling in the penis may impact the function and structure of endothelial cells, cavernosal smooth muscle cells, and nerves, and result in ED (26).

1.5. Androgens

Emerging evidence suggests that androgens exert a direct effect on the penis to preserve erectile function. Androgens maintain the structure of the corpora cavernosa, regulate growth and differentiation of vascular smooth muscle cells, and upregulate the expression/activity of constitutive NOS isoforms and PDE5 in the penis. The latter effect has been proposed to be a homeostatic mechanism that maintains a relatively constant ratio of critical enzymes for penile erection. Androgens also promote the differentiation of pluripotent stem cells into smooth muscle cells and inhibit their differentiation into adipocytes. Their action in the penis involves both genomic and nongenomic mechanisms. Long-term genomic mechanisms are mediated by the activation of classic cytosolic receptors. The mechanism of rapid nongenomic, direct vasodilatory effects of androgens are poorly defined, but may involve membrane receptors, ion channels, and intracellular signaling molecules such as cAMP and cGMP (27).

Androgens regulate central, as well as peripheral neural mechanisms of penile erection. Centrally, penile erection elicited by stimulation of the medial preoptic area has been shown to be testosterone dependent. Peripherally, testosterone maintains the integrity of the dorsal and cavernous nerves of the penis. Androgen deprivation is associated with penile tissue atrophy, endothelial dysfunction, alterations in the nerve structures of the penis, and ED (27).
2. ERECTILE DYSFUNCTION (ED)

ED has been defined by the National Institutes of Health as the inability to achieve and maintain a penile erection adequate for satisfactory sexual intercourse. It is a significant health problem affecting approximately 150 million men worldwide, and is predicted to double within the next 20 years. It has been estimated that more than 80% of ED has organic origin. Organic causes of ED include hormonal changes, damage to the nerves, impaired synthesis/release/availability of neuronal or endothelial NO, impaired arterial inflow, veno-occlusion, or corporal tone.

Two major causes of ED are vasculogenic and neurogenic. The following sections present major molecular mechanisms involved in vasculogenic and neurogenic ED.

2.1. Vasculogenic ED

Accumulating evidence indicates that ED is predominantly a vascular disease. A variety of conditions that involve vascular abnormalities, such as diabetes, aging, hypercholesterolemia, hypertension, sedentary lifestyle, and cigarette smoking, are associated with the impairment of penile vascular function and vasculogenic ED in men and in a number of animal models. Vasculogenic ED refers to the derangements in the function and structure of endothelial and smooth muscle cells in the penis. Vasculogenic ED may be due to arterial or cavernosal/veno-occlusive dysfunction, resulting in reduced blood inflow into the penis, or excessive blood outflow from the penis. The inability of the endothelium to produce vasorelaxing messengers, increased vasoconstriction, and reduced endothelium-dependent vasodilatory response of smooth muscle cells, are sources for the development of endothelial dysfunction in the penis and vasculogenic ED. Endothelial dysfunction is an early stage of vascular damage, which can lead to more severe changes like atherosclerosis in the systemic vasculature and manifests clinically as coronary heart, renal, cerebral, and peripheral artery diseases. In fact, vascular ED appears to be one of the earliest signs of systemic microvascular and macrovascular diseases and may be considered an early marker for cardiovascular diseases (18).

Increases in oxidative stress contribute to the development of ED (28). Known sources of ROS in the penis include NAD(P)H oxidase (described in diabetes-hypertension), and hypercholesterolemia-associated ED; 3), and eNOS uncoupling (described in hypercholesterolemia-associated ED; 16). Decreased NO-mediated vasorelaxation in ED associated with aging, diabetes, hypercholesterolemia, and hypertension has been attributed to impaired eNOS expression (decreased or increased) and decreased eNOS activity (due to impaired eNOS phosphorylation, decreased substrate availability, or increased eNOS interaction with its negative regulator caveolin-1), upregulation of PDE5, and upregulation of the vasoconstricting RhoA/Rho-kinase pathway in the penis. Impaired growth factor and cytokine production and signaling in the penis impacts the function and structure of endothelial and cavernosal smooth muscle cells. Morphologic changes in the diseased penis involve endothelial and smooth muscle damage, smooth muscle proliferation, increased collagen deposition, and thinning of the tunica albuginea. Apoptosis and degeneration of smooth muscles, increase in connective tissue, and fibrosis in the penis all lead to corporal veno-occlusive dysfunction and ED (18). Androgen deficiency leads to reduction in eNOS and PDE5 protein expressions, damage to the endothelium with reduced number of circulating progenitor endothelial cells, endothelial dysfunction, reduction in smooth muscle content, increase in connective tissue and adipocytes, reduced veno-occlusion, fibrosis, and ED (27).

2.2. Neurogenic ED

Neurogenic ED results from any defect in neurotransmission to the smooth muscle of the penis. It can be related to trauma to nerves such as in spinal cord injury, injury of nerves as a consequence of surgeries for cancer of the prostate, bladder and colon, or is an associated element of a neurological disease such as multiple sclerosis, Parkinson’s disease, Alzheimer’s disease, stroke, and encephalitis. More commonly, neurogenic ED results from the degeneration and loss of the nerves associated with nontraumatic chronic diseases such as diabetes. Although the molecular mechanisms underlying neurogenic ED are not well understood, the principal theories include impairment in nNOS expression/function and NO bioavailability, reduced blood supply to nerve tissue, deficiency of neurohormonal growth factors, and increased oxidative stress (29).

Molecular mechanisms underlying decreased neurogenic-mediated corpus cavernosum relaxation involve disturbances in the central and peripheral systems of neurotransmission. Central neuropathy involves increased apoptosis in the hypothalamic PVN (described in aging-diabetes-associated ED) and MPOA (described in diabetes-associated ED). Neuronal loss in the brain is due to an increase in the rate of apoptosis by oxidative and nitrosative stress. The brain has relatively low levels of antioxidant defenses and high lipid content, which is highly susceptible to ROS attack. Peripheral mechanisms have been attributed to a reduction in nitrergic penile nerve fibers in the penis and decreased nNOS expression and activity, resulting in insufficient production of NO at the penile nerve terminals in response to sexual stimulation (26). Diabetes is the most common cause of peripheral neuropathy. In type I diabetic rats decreased nNOS content in the axons of nitrergic nerves, possibly due to a defect in axonal transport, is followed in more advanced diabetes by apoptosis of nitrergic nerve cell bodies. This leads to irreversible loss of nNOS content and nitrergic function and a decrease in nerve fibers. Apoptosis in nitrergic nerves has been attributed to increased oxidative stress (30). Impairment in neurotrophic and growth factor production and action may compromise penile erection, as shown in diabetic and cavernous nerve injury animal models, the latter simulating the neural injury that occurs during radical prostatectomy, a common cause of ED (26). Animal models of androgen deficiency demonstrated the impact of castration on decreased structural integrity of the penile dorsal and cavernous nerve, and depletion of nNOS in neurons of the major pelvic ganglia and cavernous and dorsal nerve in the penis (27).

3. SUMMARY AND CONCLUSION

Many advances in the understanding of basic erection physiology and pathophysiology have been made in the past decade. These advances have revealed the complexity of regulation of erectile function both under normal conditions and in association with various disease states. Multiple molecular pathways and mechanisms at the central nervous system level and at the peripheral level regulate the normal erectile response, and disturbances at any level will result in ED. The identification of the roles and mechanisms of action...
of various mediators, as well as their interactions, in normal erectile function is a major scientific development in the study of ED. However, the precise physiologic and pathophysiologic mechanisms are still incompletely known. For example, there are many unanswered questions about the role of oxidative stress and the sources of ROS in the penis, the role and regulation of RhoA/Rho kinase pathway, the factors involved in the regulation of eNOS and nNOS function, and the neurogenic mechanisms involved in erection biology. Further understanding of the molecular basis of erectile function physiologically and pathophysiologically may lead to the development of new therapeutic avenues based on targeting a specific mechanism associated with a specific ED condition.

Abstract

Penile erection is a neurovascular process which relies on a coordination of the nervous system and the vascularization of the penis, and is modulated by hormonal factors. In recent years research has increasingly focused on molecular mechanisms operating from the central nervous system to peripheral end-organ levels involved in penile erection. Major scientific advances have occurred in the study of erection physiology at the molecular level. Specific signaling pathways have been described which provide a molecular biological basis for penile erection. Basic science discoveries of nitric oxide as the main mediator of penile erection paved the way for identifying mechanisms underlying physiologic and pathophysiologic events in the penis. The balance and interactions between relaxant and contractile factors regulating penile smooth muscle tone determine penile flaccidity or erection. Defective regulation of penile erection can be associated with the impairment of the neurogenic, vascular, cavernosal, or hormonal factors.

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