Revisión

Physiology of penile vasculature: endothelium and erectile dysfunction

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ABSTRACT

Erectile dysfunction (ED) is considered as an early sign of endothelial dysfunction and cardiovascular disease. The present review gives an overview of the physiological factors involved in the regulation of penile vasculature with special regard to the role of the vascular endothelium. Sympathetic nerves maintain flaccidity and mediate detumescence through the release of the vasoconstrictor noradrenaline that down-regulates its own release and that of nitric oxide (NO). The sympathetic cotransmitter neuropeptide Y (NPY) enhances noradrenergic vasoconstriction and also elicits non-NO endothelium-dependent relaxations. α_1-adrenoceptor-mediated vasoconstriction involves Ca^{2+} influx through L-type and receptor-operated (ROC) Ca^{2+} channels, as well as Ca^{2+} sensitization mechanisms mediated by protein kinase C (PKC), tyrosine kinases (TKs) and Rho kinase (RhoK) in penile small arteries. Rho kinase (RhoK) can regulate Ca^{2+} entry in addition to its role in Ca^{2+} sensitization. Vasodilatation of penile arteries and large veins during erection is mediated by neurally-released NO and the subsequent increased arterial inflow and shear stress activates endothelial NO production through Akt-phosphorylation of endothelial NO synthase (eNOS). cyclic guanonin 3´-monophosphate (cGMP)-stimulated K^+ efflux through Ca^{2+}-activated (K_{Ca}) and voltage-dependent Ca^{2+} (K_v) channels is involved in the NO relaxant responses of penile arteries and veins, respectively. Phoshodiesterase type 5 (PDE5) inhibitors are potent vasodilators of penile arteries and increase the effects of basally released endothelial NO. Endothelium-dependent relaxations of penile small arteries include an endothelium-derived hyperpolarizing factor (EDHF)-type response which is

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impaired in diabetes and hypertension associated ED. The changes in structure and function in the penile vasculature in cardiovascular risk situations leading to ED remain to be elucidated.

Key words: Penile arteries, nerves, endothelial cells, nitric oxide, vasculogenic erectile dysfunction.

RESUMEN

Fisiología de los vasos sanguíneos peneanos: endotelio y disfunción eréctil

Se considera la disfunción eréctil (DE) una manifestación temprana de disfunción endotelial y enfermedad cardiovascular. En el presente trabajo se resumen los factores fisiológicos implicados en la regulación de los vasos sanguíneos peneanos, con especial referencia al papel del endotelio vascular. Los nervios simpáticos mantienen la flacidez y median la detumescencia a través de la liberación de noradrenalina que regula su propia liberación y la de óxido nítrico (NO). El cotransmisor simpático neuropéptido Y (NPY) potencia la vasoconstricción noradrenérgica y también produce relajación endotelial no mediada por NO. La vasoconstricción α_1-adrenérgica está mediada por la entrada de Ca^{2+} a través de canales tipo L y canales operados por receptor, así como también mecanismos de sensibilización al Ca^{2+} mediados por la proteína kinasa C (PKC), tirosina kinasas y la kinasa Rho (RhoK). La RhoK puede regular la entrada de Ca^{2+} además de su papel en la sensibilización al Ca^{2+}. La vasodilatación de las arterias y venas grandes durante la erección está mediada por NO de origen nervioso y el posterior incremento del influjo de sangre arterial y *shear-stress* activa la producción de NO endotelial a través de la fosforilación Akt de la enzima NOS endotelial (eNOS). El eflujo de K^+ estimulado por el guanosín monofosfato cíclico (GMPc) a través de canales de K^+ activados por Ca^{2+} (K_{Ca}) y por voltaje (K_v) está implicado en el efecto relajante del NO en arterias y venas peneanas. Los inhibidores de la fosfodiesterasa tipo 5 (PDE5) son potentes vasodilatadores de las arterias peneanas que incrementan los efectos del NO endotelial liberado basalmente. Las respuestas relajantes dependientes del endotelio incluyen una respuesta de tipo *factor hiperolarizante derivado del endotelio* (EDHF) en las arterias peneanas pequeñas que está alterada en la DE asociada a la diabetes y la hipertensión. Los cambios en estructura y función de los vasos sanguíneos penenanos en situaciones de riesgo cardiovascular que ocasionan DE están todavía por clarificar.

Palabras clave: Arterias peneanas, nervios, célula endotelial, óxido nítrico, disfunción eréctil vasculogénica.

INTRODUCTION

Erectile dysfunction (ED), defined as the consistent inability to achieve and maintain a penile erection sufficient for adequate sexual performance (1), is a predominantly vascular disease with a high prevalence in patients with vascular disorders such as diabetes, aging, hypercholesterolemia, hypertension, sedentary life style and cigarette smoking (2, 3). Loss of endothelium integrity and subsequent endothelial dysfunction during the latter pathological conditions plays an essential role in the pathogenesis of ED. Therefore, ED is considered as an early manifestation of systemic endothelial dysfunction and cardiovascular disease (4).

The nitric oxide (NO) pathway is of critical importance in the physiology of penile erection and also in the pathophysiology and pharmacological management of ED (4-6). The constitutive endothelial NO synthase (eNOS) and neuronal NO synthase (nNOS) isoforms are tightly regulated and produce relevant levels of NO in endothelial cells and autonomic nerve endings of the penis (4). Penile erection occurs when NO released from nerve terminals and endothelium upon sexual stimulation decreases vascular resistance and increases blood flow through cavernous and helicine arteries. This allows the filling of the sinoids, the rise in intracavernosal pressure (ICP) and the restriction of venous outflow by compression of subtunical veins against the tunica albuginea with entrapment of pressurized blood in the corpora cavernosa, which is referred to as *veno-occlusive mechanism* (5, 7, 8). ED can be considered as a three-fold process where arterial insufficiency is followed by inability to obtain tumescence due to deffective filling of the corpum cavernosum (CC) and faulty veno-occlusion (9).

Penile erection and flaccidity are ultimately regulated by the relaxation and contraction, respectively, of erectile smooth muscle, and a metabolic imbalance between contractile and relaxant factors in the CC, penile arteries and veins is one of the main causes of organic ED. The present review summarizes the research work carried out by our group for more than a decade on the mechanims underlying the contractile/relaxant processes of the penile vasculature, with special regard to the role of the vascular endothelium in the phsyiology of erection, in the pathophsyiology of

965

ED and in the action of vasoactive drugs currently used in the therapy of ED.

REGULATION OF PENILE VASOCONSTRICTION AND FLACCIDITY

Sympathetic nerves

Sympathetic adrenergic nerves are responsible for the detumescence of the erect penis and also contribute to the maintenance of penile flaccidity through the tonic release of noradrenaline (10). Noradrenaline induces contraction of CC smooth muscle and arterial vasoconstriction, which leads to the reduction of arterial inflow and to the collapse of the lacunar spaces, respectively, allowing decompression of subtunical venules and venous drainage of the corpora (5, 7, 11). Neural-released noradrenaline evokes vasoconstriction through a mixed population of postjunctional α_1- and α_2-adrenoceptors in the isolated cavernous artery (12), and only through α_1-adrenoceptors in horse penile resistance arteries (13). Noradrenaline down-regulates its own release from adrenergic nerves acting on inhibitory prejunctional α_2-adrenoceptors in intracavernous arteries (13).

Besides the mechanical restriction of the venous outflow during the *veno-occlusive* mechanism, there is evidence for an active regulation of penile veins and adrenergic stimuli induce venoconstriction through and heterogenous population of α_1- and α_2-postjunctional adrenoceptors in both circumflex (14) and deep dorsal veins (15). In the latter, noradrenergic nerve terminals penetrate deep in the smooth muscle effector layer which represents a mechanism to respond faster and more extensively to the vasoconstrictor nerve activity.

In addition to its classical vasoconstrictor role, adrenergic transmitters can also induce vasodilatation in penile arteries through β-adrenoceptors, which is related to the increased plasma adrenaline levels during erection. By acting on β_2-adrenoceptors (7, 13), adrenaline would further increase arterial inflow to the penis in connection with the increased metabolism and physical activity during sexual intercourse.

Activation of sympathetic nerves still has antierectile effects under conditions of α-adrenoceptor blockade suggesting that neuro-transmitters other than noradrenaline may contribute to the vasoconstrictor sympathetic activity in the penis (10). Neuro-peptide Y (NPY), a 36-aminoacid peptide usually colocalized with noradrenaline in sympathetic perivascular nerves, is widely distributed in penile erectile tissues with a particularly high density around helicine arteries (16, 17). Although NPY was initially suggested to have a role in detumescence (10), earlier *in vitro* studies on the contractile effects of the peptide on erectile tissue rendered controversial results (16), and *in vivo* investigations showed that intracavernous injection of NPY increased intracavernous pressure in rabbits (18). We have recently provided an explanation for this controversy between the rich presence of NPY-containing nerves in the penis and the lack of clear antierectile/vasconstrictor effects of the peptide (17). Thus, NPY has a dual facilitatory/inhibitory modulatory role on the noradrenergic vasoconstriction of horse penile resistance arteries, the ability to enhance and decrease noradrenaline-induced contractions being achieved through a heterogenous population of NPY receptors. Both Y_1 and Y_2 postsynaptic receptors are involved in the NPY-induced enhancement of noradrenaline contractions, and presynaptic inhibitory Y_2 receptors limit noradrenaline release form nerve terminals. The Y_1 receptor is a G-protein-coupled receptor that usually enhances other constrictors responses in small arteries by depolarizing arterial smooth muscle and by inhibiting cAMP-mediated relaxations (19, 20). Under conditions of Y_1 and Y_2 receptor blockade, NPY can also induce NO-independent relaxations in penile resistance arteries through atypical receptors located at the endothelium, which could account for the *in vivo* proerectogenic effects reported for the peptide (18).

Local factors regulating penile vasoconstriction

Several contractile prostanoids are locally synthesized and metabolized in penile erectile tissues (21). Whereas in CC basal pro-stanoid production is involved in the maintenance of the myogenic spontanous tone consisting of phasic and tonic contractions (21), in penile resistance arteries arterial spontaneous tone is modulated by

basal-released relaxant prostanoids (22). On the other hand, agonist-induced endothelial stimulation of both human and rabbit CC and horse deep dorsal penile veins evokes the release of contractile prostanoids (23, 24). The main receptor involved in the contractile effect of prostanoids in the human penis belongs to the PGH_2/TP type (25, 26).

Angiotensin II (AII) is locally formed in the endothelium and smooth muscle of the CC, where its contractile effects are mediated by muscular AT-1 receptors (27-29). Since AII levels are increased in the cavernous blood during detumescence (29), this peptide has been suggested to be involved in the initiation of detumescence as a consequence of enhanced sympathetic activity. However, the functional role of AII in penile arteries and veins remains to be established.

Endothelins (ETs) are potent vasoconstrictor peptides synthesized by endothelial cells that induce both contraction and ET_B-mediated relaxation of erectile tissues (30, 31). ET_A and ET_B receptors subtypes are located at penile smooth muscle and nerves, respectively (30), and infusion of ET-1 and ET-3 in the rat increases intracavernosal pressure at low doses, and produces vasoconstriction followed by decrease in pressure at high concentrations (32).

Intracellular mechanisms of penile vasoconstriction

Although elevation of $[Ca^{2+}]_i$ is considered as a classical trigger for force development, smooth muscle contraction can also be modulated through inhibition of myosin light chain (MLC) phosphatase (MLCP) by Ca^{2+}-independent mechanisms resulting in an increased MLC phosphorylation and force at constant $[Ca^{2+}]_i$, which is referred to as *Ca^{2+} sensitization* (33). Vasoconstrictor agonists binding G-protein-coupled receptors can stimulate both Ca^{2+} mobilization from intracellular stores and Ca^{2+} entry through both dihydropyridine-sensitive L-type and non L-type Ca^{2+} channels (34).

Whereas in rat and human CC, there is a significant contribution of Ca^{2+} from intracellular stores to phenylephrine-induced contractions (35), in penile small arteries, α_1-adrenoceptor- and TP receptor-mediated contractions are largely dependent on Ca^{2+} entry

through both voltage-dependent L-type and receptor-operated Ca^{2+} channels (ROCs), with a minor role for either intracellular Ca^{2+} mobilization or store-operated Ca^{2+} (SOC) entry (36, 37). A *capacitative* Ca^{2+} entry through SOCs not coupled to contraction but probably related to non-contractile functions of vascular smooth muscle cells, such as protein and gene expression, is also present in penile small arteries. Despite the large extracellular Ca^{2+} dependence of penile vasoconstriction, there are also potent mechanisms of Ca^{2+} sensitization of the contractile proteins involving several kinases such as protein kinase C (PKC), tyrosine kinases (TKs) and Rho kinase (RhoK) (37), which suggests that Ca^{2+} entry and Ca^{2+} sensitization may cooperate to elicit vasoconstriction upon α_1-adrenergic receptor stimulation. Rho A is a member of the Ras superfamily of small GTP-binding proteins and Rho kinase (RhoK) is a serine-threonine kinase which is activated by RhoA (33) and has been shown to play a key role in the physiology of erection. Thus, inhibition of RhoK in a rat *in vivo* model markedly increases intracavernosal pressure and leads to erection (38) and adeno-associated viral gene transfer of dominant negative RhoA mutant enhances erectile function (39). In human CC, RhoA-mediated Ca^{2+} sensitization contributes to smooth muscle contraction and flaccidity (40). Interestingly, we have recently shown that RhoK is involved not only in the Ca^{2+} sensitization of the contractile apparatus but also in the regulation of Ca^{2+} entry through ROC channels upon α_1-adrenoceptor activation in rat penile small arteries (41). These findings suggest that RhoK and other kinases involved in Ca^{2+} homeostasis of penile arteries represent potential therapeutic targets for the treatment of organic ED (Figure 1).

REGULATION OF PENILE VASODILATATION AND ERECTION

Accumulating experimental evidence for more than a decade now has supported nitric oxide (NO) as a central component of the major signal transduction pathway mediating penile erectile responses. Wheras the role of NO as a neurotransmiter in the reflex arc that initiates erection was early established, endothelial-derived NO is becoming increasingly recognized as a key factor in the vascular homeostasis and physiology of penile erection.
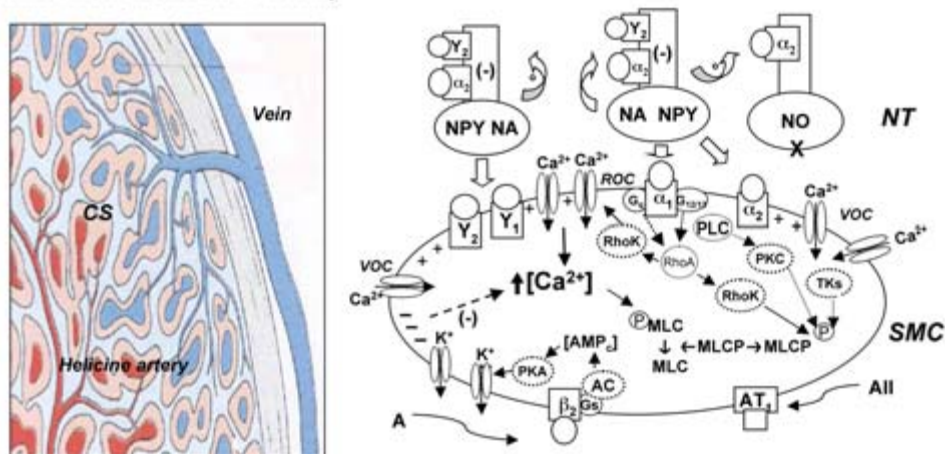
Penile vasoconstriction-Flaccidity

FIGURE 1. **Penile vasoconstriction and flaccidity.** *Noradrenaline (NA) released from sympathetic nerves acts on a_1 and a_2 postsynaptic receptors to elicit vasoconstrcition and on a_2 presynaptic receptors to down regulate its own release and that of nitric oxide (NO). Activation of a_1 adrenoceptors evokes Ca^{2+} entry through voltage.dependent (VOC) and receptor-operated channels (ROC) regulated by Rho kinase (RhoK), thus activating myosin light chain (MLC) kinase (MLCK) which phosphorylates MLC. Ca^{2+} sensitization is also activated through RhoK, protein kinase C (PKC) and tyrosine kinases (TKs) leading to phosphorylation of MLC phosphatase (MLCP). Neuropeptide Y (NPY) is coreleased with NA from sympathetic nerves and acts Y_1 and Y_2 postsynaptic receptors to enhance NA contractions and on Y_2 receptors to limit NA. Adrenaline (A) can elicit relaxation through b_2 adrenoceptors linked to adenylate cyclase (AC), increase of cAMP and activation of protein kinase A (PKA) to enhance K^+ efflux and decrease intracellular Ca^{2+} and contraction. AII: angiotensin II; NT: Nerve terminal; SMC: Smooth muscle cell.*

Non-adrenergic Non-cholinergic (NANC) NO-containing nerves

Penile erection is initiated by activation of parasympathetic nerves leading to vasodilatation of cavernous and helicine arteries and to the relaxation of trabecular smooth muscle. However, classical physiological studies carried out in several animal species and man showed that erection induced by activation of pelvic or cavernous nerves was atropine-resistant (5). The neurotransmitter involved in the erectile responses was further shown to be NO, and it is now well established that NO locally released from nerves and

endothelium is the main chemical mediator of the vasodilatation and trabecular relaxation involved in penile erection.

The catalytic production of NO requires the enzyme NO synthase, expressed in many biological tissues as 3 main isoforms: two constitutive isoforms, neuronal NOS (nNOS) and endothelial NOS (eNOS), that require Ca^{2+} and calmodulin for activity, and one inducible isoform (iNOS), that is Ca^{2+}- and calmodulin- independent and is expressed in response to inflammatory mediators (42). nNOS and eNOS are the principal isoforms involved in penile erection and are present in the nerves and endothelium of the penis, respectively (43-45). nNOS is present in the pelvic plexus and in terminal branches of the cavernous nerves innervating the trabecular smooth muscle and penile vasculature (43, 46). nNOS and vesicular ACh transporter (VAChT) are co-localized in most nerve terminals surrounding penile small arteries which suggests that ACh and NO coexist in the same parasympathetic cholinergic neurons that control penile arterial tone (44, 45).

Investigations by our group first provided functional evidence for the involvement of NO in the NANC inhibitory neurotransmission of penile resistance arteries or *helicine arteries*. Thus, the NANC relaxations elicited by electrical field simulation (EFS) were endothelium-independent, inhibited by the L-arginine analogue, N^G-nitro-L-arginine, by the NO scavenger, oxyhaemoglobin, and by inhibitors of guanylate cyclase (47, 48). Moreover, exogenous NO and nitrosothiols mimicked the vasodilatation induced by EFS (22, 47, 48), which suggests that NO or a NO-like substance released form nitrergic nerves mediates the neurogenic relaxations of penile resistance arteries.

Until recently, the defined role of nNOS and eNOS in penile erection has been a subject of debate, specially because erectile function and mating behaviour are preserved in mice lacking the genes for eNOS and nNOS (49, 50). Recently, a nNOS gene variant resulting from alternative mRNA splicing of the βnNOS in exon 1 has been confirmed as a major mediator in penile erection by using nNOS, eNOS and doubly mutant deficient mice (51).

Concerning the role of other neural mediators released from parsympathetic nerve terminals during penile erection, the classical

971

cholinergic neurotransmitter acetylcholine (ACh) relaxes CC smooth muscle and penile arteries and veins (5, 22, 24, 52), despite the fact that erection is atropine-resistant. ACh released from cholinergic nerves during erection probably has a role decreasing sympathetic adrenergic tone, though its action on prejunctional muscarinic receptors at sympathetic nerve terminals as reported in other small arteries and CC (7, 8, 53).

There is a rich presence of peptidergic nerves containing Vaoactive Intestinal Peptide (VIP) in the penis and nNOS, VIP and VAChT are co-localized in the same perivascular nerve terminals around penile arteries and in trabecular smooth muscle (44, 45). VIP induces potent relaxations in penile arteries and veins (5, 54). However, VIP antagonists do not inhibit neurogenic relaxations in penile small arteries but VIP relaxant responses are partially blocked by NOS inihibition, which suggests that VIP may have a presynaptic facilitatory role in the nitregic neurotranmission (54).

The synthesis and release of NO in the penile vasculature is modulated by several factors such as neurotransmitters, hormones, autacoids and O_2 tension. Noradrenaline from sympathetic nerves down-regulates NO release through α_2-adrenoceptors in penile resistance arteries (55). The expression and activity of NOS can also be regulated by protaglandins and PGE_1 exerts a long term up-regulation of NO synthesis by increasing the penile content of both nNOS and eNOS after repeat treatment (56).

Partial O_2 pressure (pO_2) in the blood of the CC plays a key role in the regulation of penile haemodynamics. pO_2 values are similar to those of venous blood during flaccidity and they rise to 90-100 mm Hg during erection, as a result of the increased arerial inflow to the sinuses (57). Since molecular O_2 is a substrate for NO synthesis, pO_2 regulates the ability of CC smooth muscle to relax in response to EFS of the nerves and endothelium-dependent vasodilators such as ACh, these NO-dependent responses being progressively inhibited as a function decreasing pO_2 levels (57). Furthermore, both nNOS and eNOS proteins dramatically decrease in the erectile tissue during chronic cavernosal isquemia which suggests that arterial insufficiency and subsequent exposure of erectile tissue to hypoxia impairs NOS expression and thus NO synthesis and relaxation (58).

This probably underlies arteriogenic ED induced by atherosclerosis and other arterial occlusive diseases.

Local factors regulating penile vasodilatation

Relaxant prostanoids synthesized by both smooth muscle and endothelial cells, are involved in the maintenance of penile arteriolar tone (22, 26) and in the endothelium-dependent vasodilatations of penile arteries in some animal species (54). Since PGE_1 is the most efficacious drug used in the intracavernous therapy of ED, its relaxant effects are well characterized in both arterial and trabecular smooth muscle of the penis. Thus, PGE_1 evokes endothelium- and NO-independent relaxations mediated by cAMP in horse penile resistance arteries (59, 60), this relaxant effect of PGE_1 in human arteries being correlated with its clinical effectiveness when injected intracavernously in patients (61). The vasodilator effects of PGI_2 and PGE_1 are mediated by IP and EP (EP_2/EP_4) receptors coupled to Gs proteins and to the activation of adenylate cyclase, in human penile arteries (26).

Role of the endothelium in the regulation of penile vasculature

The endothelium, located at the interphase between blood and the vascular wall, regulates the underlying vascular smooth muscle tone by synthesizing short life vasoactive mediators such as NO, endothelium-derived hyperpolarizing factor (EDHF), prostacyclin (PGI_2), AII and endothelins (ETs) (62).

Endothelial-derived NO is produced in the penis by activation of eNOS in response to both hemodynamic stimuli like *shear stress* and pulsatile stretch generated by increased blood flow, and by neurohumoral factors such as ACh, bradikynin and histamine acting on specific endothelial receptors (7, 8, 22, 24, 52). Activation of eNOS by agonists is induced by increases in intracellular Ca^{2+} resulting from activation of G-protein-coupled receptors. Ca^{2+} binds to calmodulin and this classic mode of eNOS activation by Ca^{2+}/calmodulin accounts for rapid and transient production of endothelial NO (63).

NO is basally released from the arterial endothelium, as shown by the increase in sponaneous myogenic tone after blockade of NOS in human and equine penile small arteries (22, 48). The relative contribution of endothelial NO to the agonist-induced vasodilations of the erectile tissues is variable, being more relevant in CC and large penile arteries and veins (23, 24, 48) than in the small penile resistance arteries where a non-NO non-prostanoid factor is a major component of the endothelium-dependent relaxant responses (22, 64, 65).

The physiological contribution of endothelial NO released by shear-stres to penile vasodilatation has recently been suggested by cllinical investigations demonstrating a flow-dependent arterial dilatation, measured by the changes in the cavernous artery diameter after 5 min occlusion of penile flow (66). These studies showed that flow-induced vasodilatation is strongly impaired in patients with organic ED and prsposed this measurement as a clinical test to evaluate penile endothelial function. However, the involvement of NO in the flow-dependent dilatation of penile arteries awaits further confirmation, although recent studies have elucidated the link between increased blood flow to the penis and eNOS activity, thus also clarifying the physiological interaction between endothelial and nerve-derived NO during penile erection. Thus, erection elicited by cavernosal neve stimulation is mediated by phosphatidylinositol 3-kinase (PI3-kinase) and activation of the protein kinase Akt. (67). This pathway phosphorylates eNOS at Ser-1177 thereby increasing endothelial-derived NO (68) and is responsible for sustained NO production and the maintenance of maximal erection (67, 69). The most important physiological agonist for such eNOS activation is shear stress, although several hormones and growth factors also increase the activity of PI3-K and endothelial NO production after phosphorylation of eNOS. This pathway thus explains the role and interactions nNOS and eNOS during penile erection. Neural NO–mediated vasodilatation and increased blood flow to the CC produced by parasympathetic activation during the initiation of erection leads to shear-stress mediated stimulation of the endothelial lining in penile arteries, which in turn releases NO from the endothelium and produces further vasodilatation and sustained erection.

The subcellular location of eNOS, its interaction with the protein caveolin-1 and the phophorylation state of serine and treonine

residues of the enzyme play a role in the posttranslational regulation of eNOS (63, 67, 68).

EDHF

In systemic small arteries including penile resistance arteries, there is a significant NOS- and cyclooxygenase-resistant relaxation evoked by endothelium-dependent agonists under conditions of NOS blockade, in contrast to that observed in CC where the endothelium-dependent vasodilatation evoked by ACh is blunted by blockade of NOS (22,48,63). This non-NO non-prostanoid endothelial is associated with cyclic nucleotide-independent hyperpolarization of arterial smooth muscle thereby being referred to as «endothelium-derived hyperpolarizing factor» (EDHF) (62). As reported for the systemic circulation, in both penile arteries and veins there is also a correlation between vessel size and the relative contribution of EDHF to the endothelium-dependent relaxations, the smaller the vessel the larger the component of the relaxation resistant to NOS blockade (22, 24, 48, 54).

In horse penile small arteries, we first observed that the component of the endothelium-dependent relaxations resistant to NOS- and cyclooxygenase blockade was inhibited by raising extracellular K^+ and by a combination of intermediate- and small-conductance Ca^{2+}-activated K^+ (K_{Ca}) channel blockers, thus suggesting the involvement of a hyperpolarizing factor (22). These findings were further confirmed in human penile arteries, where the EDHF-mediated relaxations were enhanced by calcium dobesilate (64). In horse penile arteries, the EDHF-type response is also markedly inhibited by ouabain which suggests an involvement of the Na^+-K^+ ATPase (22). The identity of EDHF still remains controversial and there is probably more than one EDHF (62, 70). The available experimental evidence supports three explanations for the identity and actions of EDHF: a) increased $[Ca^{2+}]_i$ triggers the synthesis of a CP450 metabolite which is essential for the EDHF-type responses, b) endothelial cell hyperpolarization is transmitted to the smooth muscle cell through myoendothelial *gap-junctions* that couple both types of cells providing a low-resistance electrical pathway; c) K^+ ions released from endothelial cells through K_{Ca} induce

975

hyperpolarization of the adjacent smooth muscle cells by activating K^+ channels and/or the Na^+-K^+ ATPase (62, 70). In penile small arteries, endothelial intermediate-conductance K_{Ca} channels have been shown to be involved in the release of a non-NO non-prostanoid factor in rat intracavernous arteries (71), although the identity of EDHF remains to be clarified.

Intracellular signalling pathways underlying penile vasodilatation

NO constitutively produced and released from nerve terminals and endothelial cells diffuses into adjacent vascular or trabecular smooth muscle cells and binds to the enzyme guanylate cyclase to increase intracellular cGMP levels and the activity of the cGMP-dependent protein kinase (PKG) (5, 6, 63). The essential role of the PKG-mediated relaxation in the erectile process has been shown in PKG-deficient mice that are unable to reproduce and have impaired relaxations in response to neural- and endothelial-derived NO (72).

Both PKG and the cAMP-dependent kinase PKA can modulate the activity of K^+ channels thereby enhancing K^+ efflux and reducing $[Ca^{2+}]_i$ to elicit vasodilatation (73,74). K_{Ca} channels are activated by intracellular Ca^{2+} and depolarization and they are involved in the maintenance of resting tone and are downstream mediators of the NO/cGMP signalling cascade in both CC and penile resistance arteries (7, 8, 22, 47, 75). Thus, the relaxations induced by NO and NO donors in horse penile resistance arteries are inhibited by charybdotoxin and iberiotoxin, and this inhibition is not further increased by blockers of PKG thus suggesting that the relaxant effect of NO is due in part to activation of large conductance K_{Ca} (BK_{Ca}) through a PKG-dependent mechanism (22, 47, 75). In horse deep dorsal penile veins, voltage-dependent K^+ channels are involved in the maintenance of basal tone and also in the cGMP-mediated NO-induced vasodilatation (24).

The NO/cGMP signalling pathway underlying penile erection involves several targets available for pharmacological intervention in ED, the most prominent identified thus far being phosphodiesterase 5 (PDE5), the enzyme which enzymatically converts cGMP to its

inactive form (76). The cGMP-specific cGMP binding PDE5 is the main cGMP catalyzing enzyme in penile smooth muscle and selective inhibitors of the enzyme are safe and well tolerated drugs in the oral therapy of organic ED (76). PDE5 is abundant in the smooth muscle layer of human penile cavernous and helicine arteries, where the functional effects of the selective inhibitor sildenafil have been characterized (77, 78). In contrast to the CC, where sildenafil produces little or no relaxation but enhances the effects of neural-released NO (79), sildenafil potently relaxed cavernous and helicine arteries (54, 77, 78). This vasodilatation was endothelium-dependent and blocked by inhibitors of NOS, guanlylate cyclase and BK_{Ca} channels which suggests that by inhibiting cGMP break-down sildenafil augments the relaxing effects of endothelial-derived NO (75, 78). These observations are consistent with studies showing that sildenafil cannot normalize the impaired endothelium-dependent responses of human penile arteries in diabetic patients with ED (65) and could explain the suboptimal responses to oral PDEs inhibitors in these patients, in whom endothelial NO availability is decreased (69).

Several agonists acting on G-protein coupled receptors, such as β-adrenoceptor agonists, relaxant prostanoids and VIP activate adenylate cyclase and increase intracellular cAMP levels. In penile small arteries, PGE_1 relaxant reponses are mediated by cAMP and activation of ATP-sensitive K^+ channels (K_{ATP}) (59,80), in contrast to CC where K_{Ca} channels are involved (81). The activity of cAMP is terminated by the cAMP-specific PDE4 and the cGMP-inhibited cAMP-specific PDE3. They have been localized in the smooth muscle layer of intracavernous and penile resistance arteries, and in the case of PDE4 also in the cytoplasm of endothelial cells lining the cavernous arteries (77). Selective inhbition of PDE4 and PDE3 evoked potent relaxations in penile resistance arteries, which suggests an enhancement of the effects of basal cAMP production in the arterial wall; the fact that relaxations evoked by the selective PDE4 inhibitor rolipram are inhibited by cycloxygenase blockade in turn indicates an involvement of PDE4 in the release and/or effects of relaxant prostanoids (59) (Figure 2).
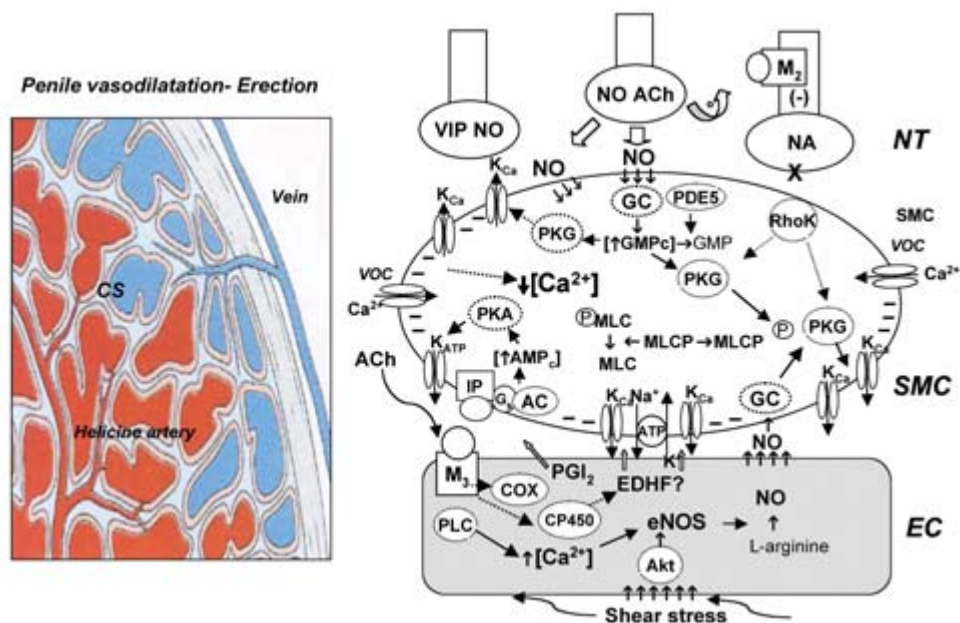
FIGURE 2. ***Penile vasodilatation and erection.*** *Nitric oxide (NO) is released from parasympathetic nerve terminals and difusses into adjacent smooth muscle to activate guanylate cyclse (GC). Increased cGMP levels activate protein kinase G (PKG) which can either stimulate Ca^{2+}-activated K^+ channels (K_{Ca}) and reduce intracellular Ca^{2+}, or phosphorylate myosin light chain phosphatase (MLCP) and reduce Ca^{2+} sensitivity. PKG can phosphorylate Rho kinase (RhoK) and thus reduce its activity. Relaxation induced by neural NO increases blood flow and shear stress on the endothelial cells thereby increasing Akt phophorylation of eNOS and NO production, and also releasing prostanoids and endothelium-derived hyperpolarizing factor (EDHF). Nerve-derived acetylcholine (ACh) can either limit noradrenaline (NA) release from sympathetic nerves through M_2 receptors or stimulate endothelial M_3 receptors to induce NO, EDHF and prostanoids release. Prostanoids activate IP/EP receptors coupled to adenylate cyclase (AC) and increased cAMP activate protein kinase A (PKA). COX: Cyclooxygenase; CP450: Cytochrome P 450; EC: Endothelial cell.; NT: Nerve terminal; PDE: Phosphodiesterase; PGI_2: Prostacyclin; PLC: Phospholipase C; SMC: Smooth muscle cell.*

ENDOTHELIAL DYSFUNCTION AND ERECTILE DYSFUNCTION

The high prevalence of ED in patients with cardiovascular disorders such as aging, hypercholesterolemia and hypertension, in

which there is endothelial dysfunction and reduced NO bioavailability (2-4), suggests the essential role of the vascular endothelium in the physiology of penile erection, as well as its importance in the pathogenesis of ED.

Mechanisms that may be related to the endothelial dysfunction and to the loss of NO bioavailability during vasculogenic ED include decreased eNOS expression and activity, dysregulation of eNOS phosphorylation, increased NO scavenging by reactive O_2 species (ROS), eNOS uncoupling, decreased levels of eNOS cofactors and substrate and increased interaction with contractile signalling pathways (63). Decreased erectile responses associated with impaired endothelium-dependent vasodilation and eNOS expression/activity in CC have been demonstrated in experimental animal models of diabetes, hypercholesterolemia and hypertension (63, 82-84). However, little is known about the impaired function of the penile vasculature and recent evidence suggests that arterial insufficiency precedes the structural and functional changes in CC leading to ED. On the other hand, whereas in CC there is a functional impairment of the NO-mediated endothelium-dependent responses during vasculogenic ED (82-84), endothelial abnormalities also include reduced EDHF-type relaxations in penile small arteries from diabetic men and from a rat model of renal hypertension (65, 85). These observations suggest the need to investigate the altered functional responses associated to cardiovascular risk situations leading to ED in penile arteries and veins.

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