Nitric oxide and penile erection: Is erectile dysfunction another manifestation of vascular disease?

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Abstract

There is convincing evidence that the prevalence of erectile dysfunction is increased among men with ischaemic heart disease. This association may be attributed to the fact that both erectile dysfunction and ischaemic heart disease share similar risk factors (e.g., hypertension, dyslipidaemia, diabetes and smoking). Nitric oxide (NO) activity is adversely affected, in penile and vascular tissue, by these risk factors. It is therefore not surprising that a defect in NO activity is thought to play a role in the pathogenesis of both erectile dysfunction and ischaemic heart disease. We consider this evidence and propose that defective NO activity provides a unifying explanation for the association between these two conditions. Further research in this area may improve our understanding of the pathogenesis of cardiovascular diseases as a whole.

Keywords: Nitric oxide; Erectile dysfunction; Diabetes mellitus; Hypercholesterolaemia; Hypertension; Cardiovascular risk factors; Endothelin

1. Introduction

Penile erection is a haemodynamic process, involving increased arterial inflow and restricted venous outflow, coordinated with corpus cavernosum smooth muscle relaxation [1]. Although this process is generally accepted to be under neuroregulatory control [1–13], biochemical mediators released locally from the endothelium and/or smooth muscle also participate in initiating and maintaining erection [14–16]. Nitric oxide (NO), which is produced both in cavernosal nerves and endothelium, has recently been recognized to play a key role in the physiology of penile erection [1–16].

In this review we consider the evidence showing that men with ischaemic heart disease have a high prevalence of erectile dysfunction. We also consider that this association may relate to the fact that the same risk factors (e.g., hypertension, dyslipidaemia, diabetes and smoking) predict both erectile dysfunction and vascular disease.

The association between erectile dysfunction and ischaemic heart disease raises the important question of whether vasculogenic erectile dysfunction is yet another manifestation of atherosclerosis. Impaired NO activity may provide a unifying explanation for such an association.

2. NO and penile erectile physiology

There is convincing evidence that during erection the local release of NO and/or related factors produces relaxation of the corpus cavernosum [1–16]. Nonadrenerg-
nic, noncholinergic (NANC) nerve-mediated NO release appears to be the most important with respect to cavernosal smooth muscle relaxation [2–13] (Fig. 1). However, the erectile process may, at least in part, be acetylcholine (ACh)-mediated in the human and rabbit corpus cavernosum [14–16].

The innervation of the corpus cavernosum and the endothelium may both be sources of NO. Using an antiserum to the constitutive NO synthase (NOS) found in rat cerebellum, Burnett et al. [16] demonstrated staining of the pelvic plexus and the axonal processes forming the cavernous nerve. The nerve plexus of the adventitia of the deep cavernosal arteries and the neuronal processes in the sinusoids and the periphery of the corpora cavernosa were prominently stained. Dorsal penile and cavernosal arteries stained for NOS both in the adventitial and endothelial layers, although endothelial staining was faint in the cavernosal vessels. Alm et al. [17] using antiserum produced in rabbits against a COOH-terminal fragment of the rat cerebellar NOS, confirmed these findings. NOS was also identified [18] in the cavernous nerves and their terminal endings within the corpora cavernosa, in the branches of the dorsal penile nerves and in nerve plexuses in the adventitia of the deep cavernous arteries. Keast [19], using nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase staining to demonstrate NOS in the rat penis, found the enzyme both in endothelial cells lining many blood vessels and within the cavernous spaces.

The NOS demonstrated in rat and rabbit corpus cavernosum was shown to display substantial activity, as monitored by the ability to convert [3H]-arginine to [3H]-citrulline [18,20]. In rabbit corpus cavernosum, the enzyme present was a cytosolic constitutive isoform of NOS [20], similar to that found in brain neuronal tissue. The endothelium-derived NOS (eNOS) is primarily membrane-bound [21]. This suggests that the most important source of NO in penile tissue is neuronal.

Analouges of L-arginine, like N⁶-monomethyl-L-arginine (L-NMMA) and N⁶-nitro-L-arginine methyl ester (L-NAME), effectively inhibit NOS. In vitro, these agents inhibit the relaxation of cavernous tissue caused by electrical field stimulation (EFS) and muscarinic receptor stimulation [2–4,6,9,11,13]. This inhibitory effect was partially counteracted by exogenous L-arginine.

N⁶-nitro-L-arginine (L-NA) augmented the contractions induced by noradrenaline but had little effect on resting

![Proposed mechanism for neuronal nitric oxide (NO) synthesis and action in the corpus cavernosum.](image-url)
intracavernosal pressure [25]. In contrast, the intracavernosal findings is that these transgenic mice retain an allele for stimulate adenylate cyclase activity induced any change in NO and / or other mediators. Another explanation for these cGMP pathways revealed that neither cAMP nor drugs that collaborative or compensatory effects of other sources of anaesthetized rats, intravenous L -NA inhibited erection localized to the vascular and sinusoidal endothelium of activating guanylate cyclase in vascular and trabecular NOS content, activity and erectile responses in the rat and mRNA, with nNOS operates locally as a post-ganglionic neurotransmitter of clari®cation.

Therefore, there is convincing experimental evidence for mice has been demonstrated by immunolocalisation studies and its synthesis and release from nerve terminals, NO the penile NO pathway. Androgen deprivation reduces NANC-mediated penile erection [1±13,20,24±30]. Follow- Several studies have assessed the role of androgens on clari®cation.

relaxation induced by EFS can be enhanced by selective inhibition of cGMP phosphodiesterase, [2,3,7,8], supporting the view that neuronally-released NO acts by stimulation of soluble guanylate cyclase activity. In rats, the intracavernous injection of drugs that act on the adenosine-3’5’-cyclic monophosphate (cGMP) and cGMP pathways revealed that neither cAMP nor drugs that stimulate adenylate cyclase activity induced any change in intracavernosal pressure [25]. In contrast, the intracavernosal injection of cGMP and SNP caused dose-dependent changes in intracavernous pressure that could be inhibited by methylene blue (a guanylate cyclase inhibitor). It was therefore concluded that cavernous smooth muscle relaxation in the rat is mediated by activation of guanylate cyclase and that the cAMP system only plays a minimal role [25].

In vitro results obtained from isolated penile tissue strips suggest that the local neuronal L-arginine/NOS system is essential for normal erection. There is accumulating in vivo data to support this view. In anaesthetized rabbits, erections induced by stimulation of the cavernous nerves could be dose-dependently inhibited by the intracorporeal injection of L-NA (an inhibitor of NOS activity) [5]. In anaesthetized rats, intravenous L-NA inhibited erection induced by the stimulation of the cavernous nerves [16]. Therefore, there is convincing experimental evidence for the assumption that neurogenic NO is an important mediator of penile erection.

to date, the majority of studies support the concept that NO derived from the autonomic innervation of the penis operates locally as a post-ganglionic neurotransmitter of NANC-mediated penile erection [1–13,20,24–30]. Following its synthesis and release from nerve terminals, NO activates guanylate cyclase in vascular and trabecular smooth muscle. The increased intracellular accumulation of cGMP is then believed to cause corporeal smooth muscle relaxation via a chemical cascade. Cyclic nucleotide phosphodiesterase enzymes (PDE’s) present in the corpus cavernosum regulate the activity of cGMP [26,27]. Experimental studies have shown that both PDE III and V isoenzymes play an important role in cavernosal smooth muscle tone [26,27]. The inhibition of PDE V activity forms the basis of treating ED with sildenafil [26,27]. One putative mechanism eliciting corporeal smooth muscle relaxation involves cGMP-dependent protein kinase de-phosphorylation of myosin light chains directly or as a consequence of lowering intracellular calcium stores. NO, via effects on transcellular ion fluxes, could also influence the contractile state of corporeal smooth musculature. Recent research suggests that NO activates sodium/potassium-adenosine triphosphatase in human and rabbit corporeal smooth musculature [28,29] as well as a K+ conductive membrane hyperpolarisation pathway in rabbit corporeal smooth muscle cells [30].

NO may be derived from penile constituents other than NANC nerves. Transgenic mice have been developed in which the neuronal NOS (nNOS) gene undergoes targetted disruption and is inactivated [31]. This has advantages over pharmacological inhibition of NOS activity, because it allows the study of the NOS enzyme independent of other NOS enzymes. These transgenic mice are viable and appear to preserve erectile function [31], suggesting the collaborative or compensatory effects of other sources of NO and/or other mediators. Another explanation for these findings is that these transgenic mice retain an allele for neuronal NOS, nNOSβ [32]. The neuronal distributions of the penis in these mice were shown to retain nNOS protein and mRNA, with nNOSβ expression demonstrable in these structures [32]. The validity of this knockout model is thus open to question. Similar disruption of the eNOS gene has resulted in mice which are aggressive, fertile and resistant to the development of atherosclerosis [33]. These studies did not specifically demonstrate erectile dysfunction or normal erectile function in these transgenic eNOS mice [33]. The findings, however, suggest that eNOS does not play a major role in the synthesis of NO involved in cavernosal smooth muscle relaxation or that alternative sources of NO and/or other mediators of penile erection can compensate for the loss of the eNOS enzyme. NOS localized to the vascular and sinusoidal endothelium of penises of normal animals and neuronal NOS-deficient mice has been demonstrated by immunolocalisation studies [34]. Inducible NOS has also been shown to be expressed on rat corporeal smooth muscle cells [35] suggesting that these cells can generate NO. To what extent these isoforms participate in normal erectile function awaits further clarification.

Several studies have assessed the role of androgens on the penile NO pathway. Androgen deprivation reduces NOS content, activity and erectile responses in the rat
penis [36]. Furthermore, its replacement in castrated rats restores these effects [37]. These restorative effects were attributed to dihydrotestosterone as the active androgen [38]. Plasma androgen levels also affect NOS activity assayed in electrically stimulated erect rat penises [39].

3. NO and the predictors of erectile dysfunction

3.1. Diabetes mellitus (DM)

DM represents one of the major organic causes of erectile dysfunction, with as many as 50% of men with DM suffering from erectile dysfunction [40]. It is also well established that DM is associated with an increased incidence of vascular events. A link between the pathogenesis of erectile dysfunction and decreased local NO activity was suggested because in isolated corpus cavernosum strips from diabetic patients with ED, both neurogenic and endothelium-dependent relaxations were impaired [41]. Similar findings were also apparent in alloxan-induced DM rabbits [42]. Reduced relaxation to EFS in cavernosal tissue taken from diabetic patients with erectile dysfunction has also been demonstrated [43]. This was associated with a lack of NO production (measured as the formation of nitrite) and not to the inability of the smooth muscle to relax [43]. Further insight into the mechanisms involved was provided by studies which showed a significant increase in NOS binding sites (putative receptors) in rat cavernosum two months post-induction of DM [44]. This finding suggests that an impairment in NO bioavailability, either due to a lack of the substrate l-arginine, due to NO quenching (e.g. by advanced glycosylation end-products (AGE’S)) [45] or via inactivation by superoxide plays a role in the pathogenesis of erectile dysfunction associated with DM.

3.2. Hypercholesterolaemia

As mentioned above hypercholesterolaemia is a recognized risk factor for both vasculogenic erectile dysfunction [46,47] and ischaemic heart disease. Studies using a genetic rabbit model of hypercholesterolaemia suggest that this lipid abnormality may account for erectile dysfunction because of changes in penile endothelin receptor distribution. These changes may, in turn, influence NOS-dependent mechanisms [48]. Experiments using cholesterol-fed rabbits have demonstrated that the inhibition of NO synthesis promotes the development of atheroma-like lesions, whereas supplementation with l-arginine prevents these changes [49].

3.3. Hypertension

Hypertension is also associated with both ischaemic heart disease and erectile dysfunction [46]. This topic is discussed in detail in the sections below.

3.4. Aging

Increasing age correlates with altered NO synthesis and erectile responses in the rat penis [50]. This could be one explanation for the increasing incidence of erectile dysfunction with aging in man [50].

3.5. Radiation effects

Radiation has been shown to reduce the number of penile NOS containing nerves in the rat, possibly providing an explanation for the development of erectile dysfunction in man, post-pelvic irradiation [51].

4. Clinical applications

Initial attempts to utilize the NO pathway therapeutically involved the intracavernous administration of the NO donor, l-arginine chloride (SIN-1), a drug that releases NO non-enzymatically [52,53]. These clinical studies [52,53] showed that SIN-1 can elicit erection, but the overall response rates are inferior to other intracavernosal agents in current use, such as prostaglandin E1 and its analogues. SIN-1 injections did not produce any local discomfort or inflammatory and fibrotic reactions, supporting the interpretation that its mode of action is physiological. More recently, sildenafil, an orally active type V PDE inhibitor, has shown response rates comparable to intracavernosal agents [54,55]. Not surprisingly, this has had a major impact on the management of erectile dysfunction.

5. Erectile dysfunction and ischaemic heart disease: does defective NO activity contribute to the pathogenesis of both conditions?

As discussed above, numerous studies have shown that ischaemic heart disease and erectile dysfunction share common risk factors [46,47]. More recently, preliminary studies suggest that fibrinogen [56] and lipoprotein (a) (our unpublished results) are risk factors for erectile dysfunction as well as for ischaemic heart disease. Epidemiologically, the long-term follow up of the Massachusetts Male Aging study concluded that the risk of moderate or complete erectile dysfunction in patients with cardiovascular risk factors was 31% (higher than in an age-matched disease-free control cohort: 19.6%) [46]. It may also be relevant that the risk factors for ischaemic heart disease and erectile dysfunction behave synergistically in both conditions.

However, of equal interest are studies that show that the extent of ischaemic heart disease is related to the risk of concomitant erectile dysfunction. Thus, two studies have
shown a significant correlation between the presence of vasculogenic erectile dysfunction and clinically evident/subclinical ischaemic heart disease [57,58]. For example, Greenstein et al. [57] reported a significant correlation between erectile dysfunction and the number of coronary vessels occluded on angiography. Anderson et al. [58] showed that patients with severe arteriogenic erectile dysfunction (assessed by duplex sonography) had a 16% risk of suffering from severe, although asymptomatic, ischaemic heart disease.

Alterations in the endothelial L-arginine-NO pathway have been demonstrated in both atherosclerotic and hypercholesterolaemic coronary arteries of humans and animal models [59–63]. These studies support the concept that there is a reduction in NO bioavailability in these conditions. These findings are similar to those seen in the penile L-arginine-NO pathway and would support the concept that vasculogenic changes in the penile vascular bed in erectile dysfunction mirror those in the coronary arteries.

The role of NANC-mediated NO release in the coronary circulation is unclear, though NANC nerves have been implicated in coronary blood flow regulation [64]. Other workers have found that NANC-mediated NO production vasodilates human cerebral arteries [65], bovine basilar arteries [66] and canine superficial temporal arteries [67], supporting a regulatory role in these vascular beds.

Decreased endothelium-dependent relaxation of isolated blood vessels has been described in experimental animal models of systemic and pulmonary hypertension [68]. These findings have been ascribed to either an attenuation of NO activity or augmented elaboration of an endothelium-derived contracting factor. More definitive data for a primary role of NO in the regulation of blood pressure has been shown in a mouse model with inactivation of the eNOS gene [33]. This impairment of endothelial NO bioavailability could be one explanation why hypertension is a risk factor for vasculogenic erectile dysfunction [46]. Obviously, hypertension-related erectile dysfunction could also result from the use of certain antihypertensive agents (e.g. thiazides or β-blockers).

Another link between erectile dysfunction and ischaemic heart disease or DM is the raised circulating levels of endothelin-1 (ET-1) [69–71]. This peptide belongs to a family of potent vasoconstrictors. To date, two major ET receptors have been identified and cloned: ET_\text{A} and ET_\text{B} [72]. ET is considered a physiological antagonist of NO [73,74] (Fig. 2). Several studies have indicated the potential importance of ET-1 in the modulation of corpus cavernosum smooth muscle tone [69,75,76]. Studies have

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**Fig. 2.** Proposed interactions between nitric oxide (NO) and endothelin-1 (ET-1) in the corpus cavernosum. This diagram is adapted (with permission) from: G.M. Rubanyi and M.A. Polokoff, Endothelins: molecular biology, biochemistry, pharmacology, and pathophysiology. Pharmacol Rev 1994;46:325–415 (solid arrows=stimulation, dashed arrows=inhibition).
suggested that ET-1 may have a role in the development of hypertension [77,78] and DM [79], both of which are cardiovascular risk factors that are also associated with erectile dysfunction [40–46,80]. The link between hypertension and erectile dysfunction is illustrated by abnormal penile vascular responses, as shown by dynamic testing (e.g., using papaverine and Duplex sonography) [80]. Hypertension acts synergistically with other vascular risk factors (e.g., DM and smoking) in terms of increasing the probability of erectile dysfunction [80]. Moreover, the plasma levels of ET-1 are higher in diabetics with erectile dysfunction than in diabetics without erectile dysfunction [79].

ET-1 potentiates the contractile response of vascular smooth muscle to other spasmogens [81–83]. Therefore, the physiological relevance of ET-1 may be related to its ability to augment the contractile responses of other vasomodulators present in the human corpus cavernosum. Hypercholesterolaemia is another cardiovascular risk factor and predictor of erectile dysfunction in both humans and animal models [46–49]. Our group has recently demonstrated, in the Watanabe rabbit model, that there is a significant decrease in ETα-receptor binding sites in corpus cavernosum tissue compared with age-matched healthy controls [48]. This reduction, in part, involved endothelial ETα receptors [48]. This receptor subtype has been shown in other vascular beds to mediate ET-1-induced vasorelaxation by stimulating NO formation [84–86]. However, the role of the ETα receptor in the corpus cavernosum remains unclear and further studies are awaited.

Hence, it appears that normal erectile function involves a delicate balance between vasodilating and vasoconstricting factors. When this balance is disrupted, erectile dysfunction may result.

Erectile dysfunction and ischaemic heart disease may not just occur in the same patients by coincidence. Consequently, medical practitioners who treat erectile dysfunction need to be aware of the possibility of underlying ischaemic heart disease and its clinical relevance in terms of ‘whole patient management’.

6. Conclusions

NO plays a major role in the physiological regulation of penile erection. NO elicits these effects through the activation of guanylate cyclase and the subsequent production of cGMP. Impaired NO activity appears to play an important role in the pathogenesis of erectile dysfunction. This impaired NO activity may be similar to that which occurs in other forms of vascular disease or in the presence of cardiovascular risk factors (e.g., smoking, dyslipidaemia, diabetes and hypertension).

The recent development of an effective, orally active, type V PDE inhibitor (sildenafil) provides a novel method of therapy for patients with erectile dysfunction. It achieves this by inhibiting the hydrolysis of cGMP, produced via the l-arginine-NO pathway.

Further research in this area is needed to determine the precise pathophysiological role of NO in this organ. This work may also provide further insights into the pathogenesis of cardiovascular diseases as a whole.

References


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