Expert Opinion on Pharmacotherapy

http://www.ashley-pub.com

Review

The role of nitric oxide in penile erection

Jon Cartledge, Suks Minhas & Ian Eardley

The Pyrah Department of Urology, St James's University Hospital, Beckett Street, Leeds, LS18 4AW, UK

The functional state of the penis, flaccid or erect is governed by smooth muscle tone. Sympathetic contractile factors maintain flaccidity whilst parasympathetic factors induce smooth muscle relaxation and erection. It is generally accepted that nitric oxide (NO) is the principal agent responsible for relaxation of penile smooth muscle. NO is derived from two principal sources: directly from non-adrenergic non-cholinergic parasympathetic nerves and indirectly from the endothelium lining cavernosal sinusoids and blood vessels in response to cholinergic stimulation. The generation of NO from L-arginine is catalysed by nitric oxide synthase (NOS). There has been controversy over the relative prevalence of endothelial or neuronal NOS within the penis of different animal species. This review examines the role of NO in the penis in detail. Established and new treatments for erectile dysfunction whose effects are mediated *via* manipulation of the NO pathway are also described.

Keywords: *erectile dysfunction, nitric oxide, phosphodiesterase inhibitors, sildenafil citrate*

Exp. Opin. Pharmacother. (2001) ***2****(1):95-107*

1. Introduction

Impotence, or male erectile dysfunction (ED), is the inability to achieve or maintain an erection of sufficient rigidity for sexual intercourse [1]. ED is a common problem. It has been suggested that approximately one in ten of all adult males experience some degree of ED [2]. The introduction of sildenafil citrate (Viagra™) to treat ED has focussed attention upon the molecular mechanisms controlling penile erection.

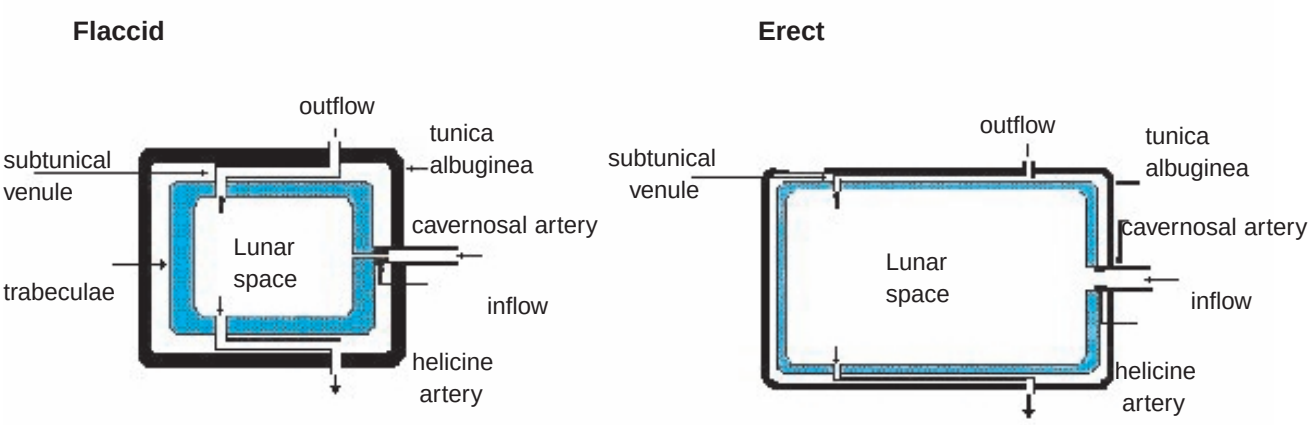
This article will summarise the current concepts of the process of penile erection and centre upon the principal agent responsible for this process, nitric oxide.

2. The mechanism of penile erection

The penis comprises a central corpus spongiosum that surrounds the urethra and expands distally to form the glans penis. Dorsally paired corpora cavernosa exist which comprise the erectile tissue. A thick fibrous sheath, the tunica albuginea, surrounds the corpora cavernosa.

Relaxation of corpora cavernosal smooth muscle is associated with penile erection, corpora cavernosal smooth muscle contraction with flaccidity [3]. Smooth muscle relaxation within penile blood vessels is as important in the process of erection as relaxation of the smooth muscle within the trabeculae of the corpora cavernosa.

Figure 1: Schematic representation of the mechanism of penile erection.
The lacunar spaces (sinusoids) of the corpora cavernosa are represented as a single unit. The trabeculae make up the walls of the sinusoids. In the flaccid state, the smooth muscle of the trabeculae is held in a state of tonic contraction that allows free venous drainage of the lacunar spaces through the subtunical venules. Following dorsal nerve stimulation relaxation of the smooth muscle in the inflow, arterial vessels increases arterial supply to the lacunar spaces. In conjunction with relaxation of the trabecular smooth muscle, the spaces expand compressing the venules against the tunica albuginea, reducing outflow. Adapted from [1].



A balance exits between smooth muscle contraction and relaxation, which is controlled primarily by a complex interplay of autonomic neurotransmitters [4]. A secondary mechanism, the vascular endothelium, can also influence smooth muscle tone [5].

A simplified summary of the haemodynamic process of erection is given in **Figure 1**.

The stimulus for erection arises either locally in the penis (reflexogenic) or centrally in the CNS (psychogenic), often synergistically [6].

Centrally originating or psychogenic erections are initiated by audio-visual, olfactory or auditory stimuli, or by fantasy. Studies in animals have highlighted the importance of the hypothalamus and specifically the medial pre-optic area of the hypothalamus in the central initiation of erections [7,8]. Descending influences lead to erection by inhibition of sympathetic and stimulation of parasympathetic spinal output.

Reflex erections result from tactile stimulation of the penis. Afferent impulses are carried in the somatic sensory fibres of the dorsal nerve of the penis that activate pro-erectile parasympathetic outflow. This reflex may be modulated by supraspinal influences [9] but is not dependent upon them [10].

Nocturnal erections, which occur 4 - 6 times per night in all normal men, are associated with REM sleep and are almost certainly centrally mediated, but their mechanism of generation is unknown [6].

Normal erectile function is, therefore, dependent upon the somatic, sympathetic and parasympathetic nerve supply to the penis.

The sympathetic nerve supply to the penis, derived from the spinal cord between the 11th thoracic and 2nd lumbar segments in man, is responsible for maintenance of the flaccid penis and the induction of detumescence following erection. Noradrenaline, acting on α-adrenoceptors, is the most important sympathetic neurotransmitter in the mammalian penis [4].

The pro-erectile parasympathetic nerve supply originates in the lumbosacral spinal cord, depending upon species - in man the cells of origin range from the 2nd to the 4th sacral segment [11]. Acetylcholine is the neurotransmitter responsible for most postganglionic parasympathetic neurotransmission [12]. Cholinergic nerves have been identified within the cavernosal nerve and the smooth muscle of the corpus cavernosum and spongiosum [13-16]. Acetylcholine may modulate the adrenergic contractile stimulus to cavernosal smooth muscle, promoting tumescence and erection [17] but its principal action is upon the endothelium of vascular and sinusoidal tissue to liberate NO.

A further group of autonomic nerves supply the penis; non-adrenergic non-cholinergic nerves (NANC) promote cavernosal smooth muscle relaxation and erection. The principal NANC neurotransmitter is NO, and this will be discussed in detail later. A second

group of NANC nerves, in which vasoactive intestinal peptide (VIP) is a neurotransmitter, also supply penile tissues, often co-localised with NO-containing neurones [18,19]. VIPergic nerves may modulate the action of other neurotransmitters, such as noradrenaline and NO [20], or act *via* the liberation of prostanoids [21]. A detailed discussion of the role of prostanoids in the penis is beyond the scope of this article and will be the subject of a subsequent review.

3. NO

It is clear that cavernosal smooth muscle relaxation is central to the process of penile erection. Marked similarities exist between the function of penile and vascular smooth muscle, indeed many techniques employed in the examination of penile tissue are adapted from studies performed on vascular tissue.

3.1 Endothelial derived relaxing factor

In 1980, Furchgott and Zawadzki published the first report highlighting the importance of the vascular endothelium in acetylcholine-mediated smooth muscle relaxation [5]. Using an *in vitro* preparation of strips and rings of rabbit aorta they demonstrated that following contraction with noradrenaline the tissue relaxed in a dose-dependent manner to acetylcholine at low concentrations. Destruction of the intimal, but not the adventitial, surface of the aortic tissue blocked the relaxation response to acetylcholine. Destruction of the endothelium had no effect on the contractile response of the tissue. The placement of strips of aorta with intact endothelium next to strips with denuded endothelium allowed the stimulation of the former and the measurement of tension changes in the latter. This experimental set up demonstrated that an active substance was transferred from a donor to recipient and established the importance of an endothelial derived relaxing factor (EDRF) in vascular smooth muscle relaxation [22].

3.2 Endothelial NO

NO was known to be a potent vascular smooth muscle relaxant [23] that acted *via* the accumulation of cGMP, akin to EDRF [24]. Further hints suggesting the identity of EDRF came from the experiments of Martin *et al.* who were able to demonstrate that both EDRF and glycerine trinitrate relaxed vascular smooth muscle; the effects of both could be blocked by haemoglobin and methylene blue [25]. Organic nitrates exert their physiological effect by a non-enzymatic production of NO and activation of guanylate cyclase [26]. Subsequently, NO was confirmed as the molecule responsible for the actions of EDRF in vascular tissue [27-29].

Incubation of endothelial cells for 24 h in the absence of L-arginine resulted in a reduced production of EDRF. This effect was reversed in the presence of L-arginine, but not D-arginine. This suggested that endothelial cells synthesise NO directly from L-arginine [30]. Support for this observation came from Schmidt *et al.* who established that L-canavanine, an inhibitor of L-arginine utilising enzymes blocked the production of NO formation and release from endothelial cells [31]. The enzyme responsible for the conversion of L-arginine to citrulline and the generation of NO is NOS.

Three isoenzymes of NOS exist, encoded by different genes on chromosomes 12, 17 and 7. Isoform III, also referred to as endothelial or eNOS, is responsible for the production of NO in endothelial cells [32,33].

The generation of NO from L-arginine is dependent on the presence of oxygen as a co-substrate and NADPH as a co-factor. eNOS is a Ca^{2+}/calmodulin-dependent enzyme that produces a low concentration of NO at resting levels of Ca^{2+} and increased levels of NO at raised levels of Ca^{2+} [34,35].

Within the membrane of endothelial cells are specialised signal-transducing regions, plasmalemmal caveolae; eNOS is preferentially localised at these sites [36]. The major constituent proteins of these regions are caveolins that are able to inhibit the function of eNOS. The formation of a reversible complex between caveolin and eNOS attenuates the function of eNOS, probably by blocking the activation of eNOS by calmodulin [37]. The association and dissociation of eNOS with caveolin is probably regulated by acetylcholine, in addition to calcium. Carbachol, a muscarinic agonist, has been shown to promote the dissociation of caveolin and eNOS in cultured endothelial cells, activating the enzyme [38].

3.3 Neuronal NO

Within the cerebellum, the existence of an agent with similar properties to EDRF has been proposed. Glutamate was known to stimulate the production of large quantities of cGMP by liberating a second messenger with properties similar to EDRF. Studies on cerebellar tissue suggested that the second messenger was a Ca^{2+}-dependent agent that was effective *via* the

activation of guanylate cyclase [39]. Subsequently, an enzyme, similar to one isolated in vascular tissue, catalysing the conversion of L-arginine to citrulline and releasing NO was described in cerebellar tissue and identified as NOS [34,40]. To identify NOS, Bredt and Snyder assayed the conversion of [3H]-arginine to [3H]-citrulline by a purified enzyme extracted from homogenised rat cerebellum. They demonstrated that cerebellar NOS activity, like eNOS, was dependent on the presence of NADPH and Ca^{2+}. The identification of an antibody to NOS enabled Bredt *et al.* to show a clear association between NOS and specific nerve fibres within the brain, but in addition to staining neural tissue the antibody also highlighted the presence of NOS in vascular endothelial cells [34]. Immunohistochemical examination of rat tissue identified a widespread distribution of the same isoform of NOS. In addition to a CNS localisation, NOS was also present in the spinal cord, sympathetic ganglia, adrenal glands, in epithelial cells of the lung, uterus and stomach, in macula densa cells of the kidney and in pancreatic islet cells [41].

Autonomic innervation of smooth muscle is able to generate a relaxant response that is not mediated by adrenergic or cholinergic neurotransmitters. There is good evidence from functional studies that the NANC neurotransmitter responsible for this effect is NO [29]. *In vitro* studies performed on arteries harvested from a bull's penis demonstrated a response to electrical field stimulation that was mimicked by a substance, inhibitory factor, derived from the bovine retractor penis muscle. The authors suggest that this substance may be the neurotransmitter responsible for NANC-mediated vasodilatation [42]. The inhibitory factor isolated from bovine retractor penile smooth muscle was able to relax strips of rabbit aorta *in vitro*, mimicking the effects of EDRF. Stripping the endothelium from the aorta in this model did not alter the response to the inhibitory factor, whereas methylene blue did. This suggests that the action of the inhibitory factor is not mediated *via* generation of EDRF but is mediated through guanylate cyclase activation, akin to NO [43]. This inhibitory factor was identified as a member of the nitrite family that could be activated by acidification to generate NO [44]. The addition of the NOS inhibitors L-NMMA and L-NOARG inhibited NANC mediated relaxation of both bovine retractor penis and rat anococcygeus muscles, supporting a role for NO as a neurotransmitter [45].

By using inhibitors with a differing specificity for endothelial- and neuronal-derived NOS, Guilmard *et al.* were able to demonstrate that the proportion of NO derived from either NANC nerve terminals or endothelium depended on the tissue examined [46]. Certainly, NANC-derived smooth muscle relaxation has been demonstrated in the intestine, lower urinary tract and penis [47,48].

3.4 The action of NO

NO, generated from L-arginine by endothelial or neuronal NOS is a small molecule with a half-life of 3 - 5 seconds [48]. NO diffuses rapidly out of its cell of origin, either endothelial cells or nerve terminals, faster than reactions responsible for its breakdown, will proceed and traverse cell membranes as readily as carbon dioxide or oxygen. This rapid diffusion allows the accumulation of the effect of NO generated from multiple cells, or nerve terminals [49].

NO acts by stimulation of the intracellular enzyme guanylate cyclase. Within guanylate cyclase, a molecule of ferrous haem is responsible for binding NO and activating the enzyme [50]. The haem component of guanylate cyclase is also capable of binding carbon monoxide, but not oxygen; activation by NO is 30-fold more efficient than carbon monoxide [51]. Both α and β haem subunits within guanylate cyclase are necessary for NO binding, following which, the enzyme binds GTP to convert it to the active form, cGMP. This catalytic conversion is dependent on the presence of divalent cations, Mn^{2+}, Mg^{2+} and probably Ca^{2+} [52]. cGMP induces relaxation of smooth muscle by the activation of an enzyme cascade, mediated through intracellular calcium levels. cGMP-dependent protein kinases, PKG and PKA, cause a phosphorylation of a protein, phospholambin. Phospholambin normally inhibits calcium pumps, within the cell wall and sarcoplasmic reticulum, thereby maintaining a high level of intracellular calcium and smooth muscle contraction. Phosphorylated phospholambin is inactive, calcium pumps, therefore, become active and intracellular calcium levels are reduced, allowing smooth muscle to relax [53].

An additional intracellular second messenger system, cAMP, exists. Activated by prostanoid stimulation cAMP might modulate the effects of cGMP and *vice versa*, probably at the level of cAMP- and cGMP-dependent protein kinases [54]. Evidence for this 'cross talk' is derived from studies in vascular tissue, there is as yet no data confirming this intracellular interaction in penile tissue.

Two mechanisms limit the action of NO *via* guanylate cyclase. Firstly, in the presence of GTP and Mg^{2+}, NO freely dissociates from guanylate cyclase, which results in an effective half-life of action of approximately 5 seconds [55]. Secondly, a family of enzymes, the phosphodiesterases (PDEs) regulates the intracellular cyclic nucleotide second messenger signalling system by catalysing the breakdown of active cGMP to inactive GMP [56].

Free NO is a highly reactive molecule that may undergo a number of non-enzymatic reactions. Louis Ignarro described seven potential reaction profiles for the destruction of NO *in vitro* [57]. It is likely only three reactions are important *in vivo*; the reaction with, and activation of, guanylate cyclase, its destruction by reaction with oxyhaemoglobin and its transformation to peroxynitrite by the reaction with superoxide anions [49].

The intracellular action of NO is mediated by guanylate cyclase catalysing the activation of GTP, following the binding of NO to a ferrous haem region of the enzyme. NO may also bind to ferrous haem contained within haemoglobin. The addition of free oxyhaemoglobin to an *in vitro* preparation of rabbit aorta and rat penis impairs endothelium-dependent relaxation, as does the addition of the guanylate cyclase inhibitor methylene blue, which suggests that the effect of oxyhaemoglobin is mediated *via* NO inhibition [58,59]. The reaction product of haemoglobin and nitric oxide, nitrosyl haemoglobin, not only forms rapidly but is also proportional to the density of haemoglobin and is highly stable [60].

Superoxide anions are highly reactive free radicals with many actions, including toxic mediation following injury, mutagenesis and amplification of the inflammatory response. Additionally, they react rapidly with NO to produce peroxynitrite, which subsequently generates inactive nitrogen dioxide. A series of enzymes, known as superoxide dismutases, exist which catalyse the conversion of superoxide, by protonation, to hydrogen peroxide, protecting NO from oxidation. Thus, superoxide dismutase is able to mediate the reaction between NO and superoxide, an extra control of the concentration of NO [61].

3.5 NO in the penis

It is known that the vasodilator mediator NO can be produced by the sinusoidal endothelium of the corpus cavernosum as well as by the penile nerves. In man it is generally accepted that NO derived from both these sources plays an important role in the relaxation of cavernosal smooth muscle that is necessary for penile erection, although the relative contribution of each source of NO is unclear [62,63]. Although NO is considered the most important agent responsible for penile smooth muscle relaxation, other compounds, i.e., endothelins and VIP, have been proposed as either contributors or modulators of this response. Their actions are beyond the scope of this article; a future review will explore the actions of endothelins and prostanoids on the penis in detail.

3.5.1 Endothelial NO in the penis

The presence of endothelial-derived NO in the mammalian penis is demonstrated by immunohistochemical and functional data. Kim *et al.* took corpora cavernosal tissue from human and rabbit penises and mounted it *in vitro* for measurement of isometric tension [63]. Tissue from both species contracted in response to noradrenaline and relaxed in a dose-dependent manner to acetylcholine. This effect was reduced by an inhibitor of NOS (L-NMMA) and blocked by both oxyhaemoglobin and methylene blue. After disruption of the endothelium by mechanical stress, or perfusion with detergents, the relaxation response seen to acetylcholine was lost. It appears, therefore, that acetylcholine exerts its relaxant effect within the penis, the same way as in vascular tissue, by the generation of NO from the endothelium. Functional data are also available to support a similar role for acetylcholine in the dog [64,65] and the mouse [66]. In a group of mice bred genetically deficient in nNOS mating responses and *in vitro* experiments demonstrate a continued ability to develop erections. Immunohistochemical studies of the corpus cavernosa from these animals reveal the presence of eNOS localised in both the sinusoidal endothelium and vasculature. In the genetically nNOS deficient mice a significantly higher concentration of eNOS was identified than in control animals at both these locations [48].

Penile nNOS (PnNOS), a variant of nNOS, has been identified as two distinct isoforms, a long and short protein, in the penis. The presence of PnNOS has been confirmed in tissue derived from the breed of nNOS negative mice that were used in the experiments of Burnett *et al.* It is possible that the erections observed within this group of animals may still be secondary to the release of neuronal-derived NO, albeit, a different isoform and the over-expression of

eNOS is only partly responsible for the maintenance of the observed erectile response [67].

Electron microscopic immunohistochemical studies have also demonstrated the presence of eNOS in human penile tissue. Using high magnification microscopy, Stanarius *et al.* were able to show that eNOS expression was localised in the endothelium lining the lacunar spaces of corpus cavernosal sinusoids and the small helicine arteries [68]. Immunohistochemical staining of cavernosal tissue from normal and impotent men examined under low power microscopy confirmed the presence of eNOS less specifically within cavernosal smooth muscle cells [69]. Two other groups, using similar techniques have demonstrated the presence of eNOS within smooth muscle cells derived from human corpora cavernosa. Bloch *et al.* used electron microscopic immunohistochemical localisation of eNOS to reveal a wide distribution within both cavernosal smooth muscle cells and the endothelium of penile vessels [70]. Rajasekaran *et al.* confirm these immunohistochemical findings but by the use of a reverse transcriptase polymerase chain reaction (RT-PCR) additionally report that cavernosal smooth muscle cells express mRNA that encodes eNOS [71].

In the rat, there is conflicting evidence over the presence of eNOS that has hampered the acceptance of this animal as a good model of erectile function. There is no functional data available to support the role of endothelial-derived NO in relaxation of cavernosal smooth muscle in the rat penis. Dail *et al.* examined the corpora cavernosa of Sprague-Dawley rats and were unable to find evidence of eNOS immunohistochemical staining in the endothelium of the sinusoids, although they were able to confirm its presence in the endothelium of vessels supplying the corpora [72]. In more recent studies, by using RT-PCR in addition to immunohistochemistry, a clear demonstration of the presence of eNOS within the endothelium lining the sinusoidal spaces of rat corpora cavernosa has been presented and supported by quantitative data, which reports greater concentrations of eNOS than nNOS at this site [73]. The presence of an endothelial source of NO is supported by the radiographic studies of Sullivan *et al.* Although this group did not demonstrate the direct presence of eNOS they were able to show that specific binding sites for NOS existed in the endothelium lining the lacunar spaces of rat corpus cavernosum [74].

It is possible that these NOS binding sites represent the caveolae described in vascular endothelial tissue by Feron *et al.* [36]. The presence of caveolae in human corpora cavernosa has been proposed following the demonstration of specific proteins, caveolin-1 and caveolin-3, in penile tissue. Caveolin-1 is capable of binding eNOS, whilst caveolin-3 is specific for nNOS binding. Caveolin-1 appears to be diffusely located within the endothelium and smooth muscle of the corpus cavernosa, whilst caveolin-3 was preferentially located in the vasculature and smooth muscle close to NADPH positive nerve fibres [75].

3.5.2 Neuronal NO in the penis

It would seem that, akin to vascular tissue, endothelial-derived NO is important in the generation of smooth muscle relaxation in cavernosal tissue. The morphological and functional significance of NO derived from NANC nerves is also well established in the penis [63,76-78].

Careful anatomical dissections of tissue harvested at the time of radical prostatectomy and early autopsy allowed Burnett *et al.* to examine the pelvic parasympathetic plexus and cavernosal nerves with immunohistochemical staining specific for nNOS and NADPH diaphorase [78]. Nerves stained positive for nNOS and NADPH were clearly described in the pelvic plexus, the neurovascular bundle that traverses the lateral border of the prostate gland and in cavernous nerves supplying the penis. Triple immunohistochemical staining of human penile tissue obtained from men undergoing penectomy for cancer has confirmed the presence of nNOS in the cavernous nerves that run within smooth muscle bundles of the crus penis. Tyrosine hydroxylase (TH) is a marker of catecholaminergic nerves; TH immunoreactive axons were identified in the same bundle as nNOS positive axons. Furthermore, in a minority of nNOS containing nerve terminals evidence of VIP co-localisation was seen [77].

Immunohistochemical studies of rat penises have clarified the identity of the inhibitory neurones transmitted within the cavernous nerve. Using a double immunolabelling technique, nNOS and VIP positive staining was identified in the same nerve fibres. Vesicular acetylcholine transporter protein (VAChT), a specific immunohistochemical stain for cholinergic nerves, was also evident within the same nerves as nNOS, suggesting that some NOS-containing penile neurones are cholinergic. A

large population of nerves were identified that stained only for nNOS and not VAChT, suggesting that many nerves are purely nitrergic [79]. Interestingly, the co-localisation of catecholaminergic nerves with nNOS positive fibres described by Tamura *et al.* was not confirmed in the rat, rather TH and nNOS immunoreactivity was seen in separate nerve fibres and terminals; albeit, nerve fibres with a similar distribution pattern [77].

Despite the immunohistochemical data that reveals the presence of nNOS within the parasympathetic nerve supply of the penis, it was *in vitro* functional studies that first proposed the importance of neuronal NO as the major inhibitory agent of corpus cavernosal smooth muscle function. Contraction of strips of corpus cavernosa mounted in an organ bath for measurement of isometric tension is seen following the addition of noradrenaline [80]. In the presence of guanethidine (an adrenergic neuronal block) and atropine (to block muscarinic receptors) a relaxant response to the application of electrical field stimulation (EFS) can be recorded. This effect is secondary to neuronal stimulation; it may be abolished by the sodium channel blocking agent tetrodotoxin [62]. In a series of *in vitro* experiments Ignarro *et al.* added inhibitors of NO formation (L-NMMA & L-NOARG), which impaired the EFS-mediated relaxation of cavernosal smooth muscle [57]. Inhibition of relaxation was also seen in the presence of methylene blue and oxyhaemoglobin. This report, the first description of the importance of NO in the penis, also measured cGMP and nitrite levels in the corpus cavernosa and determined that these were raised following stimulation of NANC nerves by EFS. Tissue harvested from male rabbits was used in all the experiments of Ignarro *et al.* A confirmation of the role of neuronal NO in cavernosal smooth muscle relaxation in man [63,81], dogs [65], rats [79] and mice [66] has also been presented using similar *in vitro* techniques.

The impairment of the action of neuronal NO by co-incubation with methylene blue implies that, as has been shown in vascular tissue, penile NO exerts its effect by activation of guanylate cyclase. This is supported by data from Hedlund *et al.* who showed that a specific soluble guanylate cyclase inhibitor, 1H-[1,2,4]-oxadiazolo [4,3-a] quinoxalin-1-one (ODQ) was also able to inhibit the rapid relaxation response to EFS [79]. A slower relaxation response was also recorded in these experiments, which was unaffected by the addition of either L-NNA or ODQ, suggesting a further agent other than NO may be in part responsible for NANC smooth muscle relaxation.

3.5.3 Intracellular effects of NO

The intracellular effect of NO in the penis is mediated by the same second messenger system as described in vascular smooth muscle. NO, acting *via* soluble guanylate cyclase, triggers both PKG and PKA to phosphorylate phospholambin, inactivating it and, hence, blocking the default action of this enzyme, which is to inhibit Ca^{2+} pumps within the sarcoplasmic and cell membrane. Following activation of the Ca^{2+} pumps intracellular free Ca^{2+} levels fall and smooth muscle relaxation follows [53]. The intracellular regulation of NO function by members of the family of PDEs has been highlighted by sildenafil citrate, a new therapeutic agent in the treatment of ED [82].

PDEs regulate the intracellular cyclic nucleotide second messenger signalling system by catalysing the breakdown of active cGMP and cAMP to inactive GMP and AMP, respectively (**Figure 2**). Nine isoforms of PDEs have been identified by protein and DNA sequencing, each with multiple subtypes and differing tissue specificity. Within the penis four isoforms, Type 2, 3, 4 and 5 are identifiable [83]. These isoenzymes are not specific for the corpus cavernosa of the penis; there is a widespread tissue distribution to include all smooth muscle cells. For example, PDE5 is widely distributed in the smooth muscle of the gastrointestinal tract and vasculature, the kidney, the nasopharyngeal mucosa, the CNS and platelets [56].

4. Pharmacotherapy of ED

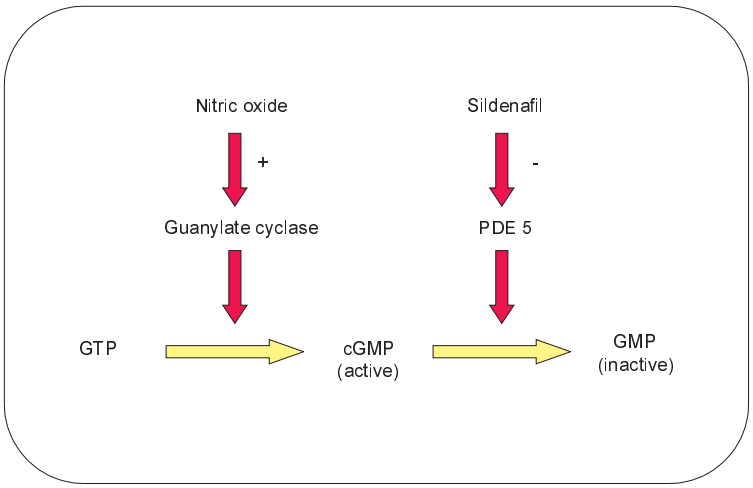
The pharmacological treatment of ED includes several agents that act *via* NO to either generate or augment an erection.

4.1 PDE inhibitors

4.1.1 Papaverine

The injection of papaverine directly into the corpus cavernosa of man will cause an erection due to non-specific inhibition of PDEs, and probably to an additional direct blockade of voltage-dependent Ca^{2+} channels [84-86]. Papaverine brings about penile erection directly without the need for sexual stimulation, and was used successfully to treat ED due to a variety of causes [84]. The incidence of serious side

Figure 2: Schematic of the intracellular action of sildenafil citrate, a specific inhibitor of phosphodiesterase 5 (PDE5).



effects, particularly priapism [87] and the development of alternative injectable therapies and eventually oral therapies, has led to a decline in its use.

4.1.2 Sildenafil citrate

The development of selective PDE inhibitors has revolutionised the treatment of ED. Since its introduction in 1998 sildenafil citrate (Viagra™) has become the first line treatment for ED. Sildenafil citrate is a selective inhibitor of the PDE5 isoenzyme [88], which has been shown to potentiate NO-mediated cavernosal smooth muscle relaxation *in vitro* [89] with an accompanying rise in cGMP levels in both animal [90] and human studies [91]. Since sildenafil citrate prevents the breakdown of available NO, sexual stimulation is required for the drug to have an effect. Data from large series of men treated for ED with sildenafil citrate confirm that this agent improves erections both qualitatively and quantitatively [92]. There have been two reported cases of priapism secondary to the use of sildenafil [93,94], more common adverse effects include headaches (12.8%), facial flushing (10%) and dyspepsia (5%) [92]. The most significant interaction of sildenafil is with donors of NO, such as nitrates, used in the treatment of angina. In combination, a sudden fall in blood pressure may result; any patient with access to nitrates should not use sildenafil.

4.1.3 Other PDE inhibitors

Initial Phase II studies of two other selective PDE inhibitors have recently been reported [95,96]. Both IC351 (ICOS Corporation) and BAY 38-9456 (Bayer AG, Cologne) are inhibitors of PDE5, and like sildenafil, are superior to placebo in the treatment of ED. A further PDE5 inhibitor, T-1032 (Tanabe Seiyaku Co., Japan), is in development, only preliminary data are available describing animal studies that suggest it may also be effective in man [97].

The specific PDE4 inhibitor, rolipram, potentiates the effects of intracavernosal prostaglandin E1 when given concomitantly, indicating that this isoenzyme is also present in human cavernosa [98]. Minimal data are available on the therapeutic effects of rolipram.

4.2 L-Arginine

It is clear from *in vitro* studies that L-arginine increases the relaxant response of cavernosal smooth muscle to EFS [99]. Oral administration of L-arginine in men with ED causes an observed [100] and significant increase in the generation of penile erection [101]. L-Arginine is not yet widely used clinically.

4.3 Gene therapy

A report describing the administration of a virus containing the eNOS gene to aged rats may point the way to the future of ED therapy. Champion *et al.* injected a recombinant adenovirus that contained the eNOS gene directly into the corprora cavernosa of rats. After 24 h, erectile responses were measured in anaesthetised animals and cGMP and eNOS activity measured in penile tissue. Both eNOS gene expression and cGMP levels were higher in the penises of

animals that had received the intracavernosal injection of virus. Functional studies revealed that infection with the eNOS gene-containing virus caused a significant increase in cavernosal pressure secondary to neuronal stimulation, acetylcholine injection and, interestingly, to administration of the PDE5 inhibitor zaprinast [102].

5. Expert opinion

To summarise, the process of penile erection is secondary to the relaxation of corpora cavernosal smooth muscle. NO released directly from NANC nerve terminals or from the endothelium of cavernosal sinusoids, in response to cholinergic stimulation, is largely responsible for mediation of this relaxation response; species differences may affect the relative proportion of each source of NO. NO acts primarily *via* an intracellular second messenger, cGMP, to relax smooth muscle directly; additionally it modifies the contractile response by regulation of the release of contractile catecholamines.

Despite the importance of NO it is possible that further regulatory mechanisms are important in the process of penile erection. These may include neuronal VIP, endothelial bound proteins, such as caveolins and further vasoactive substances generated by the endothelium.

Current therapy for ED augments the natural erectile response, it may be that in the future adenoviral-mediated gene therapy may supplement or replace available treatment.

Bibliography

Papers of special note have been highlighted as:
- • of interest
- •• of considerable interest

1. KRANE RJ, GOLDSTEIN I, SAENZ DE TEJADA I: **Impotence.** *N. Engl. J. Med.* (1989) **321**(24):1648-1659.
   - • An overview of erectile function.
2. SHABSIGH R, FISHMAN IJ, SCOTT FB: **Evaluation of erectile Impotence.** *Urology* (1988) **32**:83-90.
3. SAENZ DE TEJADA I, GOLDSTEIN I, BLANCO R, COHEN RA, KRANE RJ: **Smooth muscle of the corpora cavernosae: Role in penile erection.** *Surgical Forum* (1985) **36**:623-624.
4. ANDERSSON K-E, STIEF CG: **Neurotransmission and the contraction and relaxation of penile erectile tissues.** *World J. Urol.* (1997) **15**:14-20.
5. FURCHGOTT RF, ZAWADZKI JV: **The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine.** *Nature* (1980) **288**:373-376.
6. WEISS HD: **The physiology of human penile erection.** *Ann. Int. Med.* (1972) **76**:793-799.
7. MELIS MR, ARGIOLAS A, GESSA GL: **Oxytocin-induced penile erection and yawning: site of action in the brain.** *Brain Res.* (1986) **398**(2):259-265.
8. MARSON L, PLATT KB, MCKENNA KE: **Central nervous system innervation of the penis as revealed by the transneuronal transport of pseudorabies virus.** *Neuroscience* (1993) **55**(1):263-280.
9. RAMPIN O, BERNAB J, GIULIANO F: **Spinal control of penile erection.** *World J. Urol.* (1997) **15**:2-13.
   - • A review of the nerve supply governing erectile function.
10. RAMPIN O, GIULIANO F, DOMPEYRE P, ROUSSEAU JP: **Physiological evidence of neural pathways involved in reflexogenic erection in the rat.** *Neuroscience Lett.* (1994) **180**(2):138-142.
11. LUE TF, ZEINEH SJ, SCHMIDT RA, TANAGHO E: **Neuroanatomy of penile erection: its relevance to iatrogenic impotence.** *J. Urol.* (1984) **131**:273-280.
12. BLANCO R, SAENZ DE TEJADA I, GOLDSTEIN I, KRANE RJ, WOTIZ HH, COHEN RA: **Cholinergic neurotransmission in human corpus cavernosum. II. Acetylcholine synthesis.** *Am. J. Physiol.* (1988) **254**(3):H468-H472.
13. MCCONNELL J, BENSON GS, WOOD JG: **Autonomic innervation of the mammalian penis: a histochemical and physiological study.** *J. Neural Transm.* (1979) **45**:227-238.
14. BENSON GS, MCCONNELL J, LIPSHULTZ LI, CORRIERE JN, WOOD J: **Neuromorphology and neuropharmacology of the human penis, an *in vitro* study.** *J. Clin. Invest.* (1980) **65**:506-513.
15. DAIL WG, HAMILL RW: **Parasympathetic nerves in penile erectile tissue of the rat contain choline acetyltransferase.** *Brain Res.* (1989) **487**:165-170.
16. STIEF C, BENARD F, BOSCH R *et al.*: **Acetylcholine as a possible neurotransmitter in penile erection.** *J. Urol.* (1989) **141**:1444-1448.
17. HEDLUND H, ANDERSSON K-E: **Comparison of the responses to drugs acting on adrenoreceptors and muscarinic receptors in human isolated corpus cavernosum and cavernous artery.** *J. Autonom. Pharmacol.* (1985) **5**:81-88.
18. TAMURA M, KAGAWA S, KIMURA K, KAWANISHI Y, TSURUO Y, ISHIMURA K: **Coexistence of nitric oxide synthase, tyrosine hydroxylase and vasoactive intestinal polypeptide in human penile tissue- a triple histochemical and immunohistochemical study.** *J. Urol.* (1995) **153**:530-534.
19. HEDLUND P, ALM P, ANDERSSON K-E: **NO synthase in cholinergic nerves and NO-induced relaxation in the rat isolated corpus cavernosum.** *Br. J. Pharmacol.* (1999) **126**:349-360.

20. STEERS WD, MCCONNELL J, BENSON GS: **Anatomical localisation and some pharmacological effects of vasoactive intestinal polypeptide in human and monkey corpus cavernosum.** *J. Urol.* (1984) **132**:1048-1053.

21. KIM YC, KIM JH, DAVIES MG, HAGEN P-O, CARSON CC: **Modulation of vasoactive intestinal polypeptide (VIP)-mediated relaxation by nitric oxide and prostanoids in the rabbit corpus cavernosum.** *J. Urol.* (1995) **153**:807-810.

22. FURCHGOTT RF: **The role of endothelium in the responses of vascular smooth muscle to drugs.** *Ann. Rev. Pharmacol. Toxicol.* (1984) **24**:175-197.

23. GRUETTER CA, BARRY BK, MCNAMARA DB, GRUETTER DY, KADOWITZ PJ, IGNARRO LJ: **Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosamine.** *J. Cyclic Nucleotide Research* (1979) **5**:211-224.

24. FORSTERMANN U, MULSCH A, BOHME E, BUSSE R: **Stimulation of soluble guanylate cyclase by an acetylcholine-induced endothelium-derived factor from rabbit and canine arteries.** *Circ. Res.* (1985) **58**:531-538.

25. MARTIN W, VILLANI GM, JOTHIANANDAN D, FURCHGOTT RF: **Selective blockade of endothelium-dependent and glyceryl trinitrate- induced relaxation by hemoglobin and by methylene blue in the rabbit aorta.** *J. Pharmacol. Exp. Ther.* (1985) **232**:708-716.

26. FEELISCH M, NOACK EA: **Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase.** *Eur. J. Pharmacol.* (1987) **139**:19-30.

27. PALMER RMJ, FERRIGE AG, MONCADA S: **Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor.** *Nature* (1987) **327**:524-526.
- Confirmation that NO was EDRF.

28. IGNARRO LJ, BUGA GM, WOOD KS, BYRNS RE, CHAUDHURI G: **Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide.** *Proc. Nat. Acad. Sci. USA* (1987) **84**:9265-9269.
- Confirmation that NO was EDRF.

29. MONCADA S, PALMER RMJ, HIGGS EA: **Nitric oxide: physiology, pathophysiology and pharmacology.** *Pharmacol. Rev.* (1991) **43**:109-142.

30. PALMER RMJ, ASHTON DS, MONCADA S: **Vascular endothelial cells synthesize nitric oxide from L-arginine.** *Nature* (1988) **333**:664-666.

31. SCHMIDT HH, NAU H, WITTFOHT W *et al.*: **Arginine is a physiological precursor of endothelium-derived nitric oxide.** *Eur. J. Pharmacol.* (1988) **154**:213-216.

32. FORSTERMANN U, CLOSS EI, POLLOCK JS *et al.*: **Nitric oxide synthase isozymes. Characterization, purification, molecular cloning and functions.** *Hypertension* (1994) **23**:1121-1131.

33. POLLOCK JS, FORSTERMANN U, TRACEY WR, NAKANE M: **Nitric oxide synthase isozymes antibodies.** *Histochem. J.* (1995) **27**:738-744.

34. BREDT DA, SNYDER SH: **Isolation of nitric oxide synthase, a calmodulin-requiring enzyme.** *Proc. Nat. Acad. Sci. USA* (1990) **87**:682-685.
- Description of development of NOS immunohistochemistry.

35. MARLETTA MA: **Nitric oxide synthase: Aspects concerning structure and catalysis.** *Cell* (1994) **78**:927-930.

36. FERON O, BELHASSEN L, KOBZIK L, SMITH TW, KELLY RA, MICHEL T: **Endothelial nitric oxide synthase targeting to caveolae.** *J. Biol. Chem.* (1996) **271**:22810-22814.

37. MICHEL JB, FERON O, SASE K, PRABHAKAR P, MICHEL T: **Caveolin *versus* calmodulin.** *J. Biol. Chem.* (1997) **272**:25907-25912.

38. FERON O, SALDANA F, MICHEL JB, MICHEL T: **The endothelial nitric oxide synthase-caveolin regulatory cycle.** *J. Biol. Chem.* (1998) **273**:3125-3128.

39. GARTHWAITE J, CHARLES SL, CHESS-WILLIAMS R: **Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain.** *Nature* (1988) **336**:385-388.

40. KNOWLES RG, PALACIOS M, PALMER RM, MONCADA S: **Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase.** *Proc. Nat. Acad. Sci. USA* (1989) **86**:5159-5162.

41. SCHMIDT HH, GAGNE GD, NAKANE M, POLLOCK JS, MILLER MF, MURAD F: **Mapping of neural nitric oxide synthase in the rat suggests frequent co-localization with NADPH diaphorase but not with soluble guanylyl cyclase and novel paraneural functions for nitrinergic signal transduction.** *J. Histochem. Cytochem.* (1992) **40**:1439-1456.

42. BOWMAN A, GILLESPIE JS: **Neurogenic vasodilatation in isolated bovine and canine penile arteries.** *J. Physiol.* (1983) **341**:603-616.

43. BOWMAN A, GILLESPIE JS, SOARES-DA-SILVA P: **A comparison of the action of the endothelium-derived relaxant factor and the inhibitory factor from the bovine retractor penis on rabbit aortic smooth muscle.** *Br. J. Pharmacol.* (1986) **87**:175-181.

44. MARTIN W, SMITH JA, LEWIS MJ, HENDERSON AH: **Evidence that inhibitory factor extracted from bovine retractor penis is nitrite, whose acid-activated derivative is stabilized nitric oxide.** *Br. J. Pharmacol.* (1988) **93**:579-586.

45. LIU XR, GILLESPIE JS, GIBSON IF, MARTIN W: **Effects of NG-substrated analogues of L-arginine on NANC relaxation of the rat anococcygeus and bovine retractor penis muscles and the bovine penile artery.** *Br. J. Pharmacol.* (1991) **104**:53-58.

46. GUILMARD C, AUGUET M, CHABRIER P-E: **Comparison between endothelial and neuronal nitric oxide**

pathways in rat aorta and gastric fundus. *Nitric Oxide: Biology & Chemistry* (1998) **2**:147-154.

47. ANDERSSON K-E, PERSSON K: **Nitric oxide synthase and the lower urinary tract: possible implications for physiology and pathophysiology.** *Scan. J. Urol. Nephrol.* (1995) **29**:43-53.

48. BURNETT AL, NELSON RJ, CALVIN DC *et al.*: **Nitric oxide-dependent penile erection in mice lacking neuronal nitric oxide synthase.** *Mol. Med.* (1996) **2**:288-296.

49. BECKMAN JS, KOPPENOL WH: **Nitric oxide, superoxide and peroxynitrite: the good, the bad and the ugly.** *Am. J. Physiol.* (1996) **40**:C1424-1437.

50. IGNARRO LJ, DEGNAN JN, BARICOS WH, KADOWITZ PJ, WOLIN MS: **Activation of purified guanylate cyclase by nitric oxide requires heme. Comparison of heme-deficient, heme-reconstituted and heme-containing forms of soluble enzyme from bovine lung.** *Biochim. Biophys. Acta* (1982) **718**:49-59.

51. STONE JR, MARLETTA MA: **Soluble guanylate cyclase from bovine lung: activation with nitric oxide and carbon monoxide and spectral characterization of the ferrous and ferric states.** *Biochemistry* (1994) **33**:5636-5640.

52. HOBBS AJ: **Soluble guanylate cyclase: the forgotten sibling.** *Trends Pharmacol. Sci.* (1997) **18**:484-491.

53. STIEF CG, NOACK T, ANDERSSON K-E: **Signal transduction in cavernous smooth muscle.** *World J. Urol.* (1997) **15**:27-31.
• A review of the cellular mechanisms of cavernosal smooth muscle relaxation.

54. PELLIGRINO DA, WANG Q: **Cyclic nucleotide crosstalk and the regulation of cerebral vasodilation.** *Prog. Neurobiol.* (1998) **56**:1-18.

55. KHARITONOV VG, RUSSWURM M, MAGDE D, SHARMA VS, KOESLING D: **Dissociation of nitric oxide from soluble guanylate cyclase.** *Biochem. Biophys. Res. Commun.* (1997) **239**:284-286.

56. BEAVO JA: **Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms.** *Physiol. Rev.* (1995) **75**:725-748.
• Description of the location of multiple isoenzymes of PDE.

57. IGNARRO LJ: **Biosynthesis and metabolism of endothelium-derived nitric oxide.** *Annu. Rev. Pharmacol. Toxicol.* (1990) **30**:535-560.
• Review of NO.

58. MARTIN W, VILLANI GM, JOTHIANANDAN D, FURCHGOTT RF: **Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta.** *J. Pharmacol. Exp. Ther.* (1985) **232**:708-716.

59. CARTLEDGE J, EARDLEY I, MORRISON JFB: **Impairment of Corpus Cavernosal Smooth Muscle Relaxation by Glycosylated Human Haemoglobin.** *BJU International* (2000) **85**:735-741.

60. GORETSKI J, HOLLOCHER TC: **Trapping of nitric oxide produced during denitrification by extracellular hemoglobin.** *J. Biol. Chem.* (1988) **263**:2316-2323.

61. OURY TD, DAY BJ, CRAPO JD: **Extracellular superoxide dismutase: a regulator of nitric oxide bioavailability.** *Lab. Invest.* (1996) **75**:617-636.

62. IGNARRO LJ, BUSH PA, BUGA GM, WOOD KS, FUKOTA JM, RAJFER J: **Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle.** *Biochem. Biophys. Res. Comm.* (1990) **170**:843-850.
• Important description of the importance of NO in penile erection.

63. KIM N, AZADZOI KM, GOLDSTEIN I, SAENZ DE TEJADA I: **A nitric oxide-like factor mediates nonadrenergic-noncholinergic neurogenic relaxation of penile corpus cavernosum smooth muscle.** *J. Clin. Invest.* (1991) **88**:112-118.

64. TRIGO-ROCHA F, HSU GL, DONATUCCI CF, LUE TF: **The role of cyclic adenosine monophosphate, cyclic guanosine monophosphate, endothelium and nonadrenergic, noncholinergic neurotransmission in canine penile erection.** *J. Urol.* (1993) **149**:872-877.

65. HEDLUND P, LARSSON BL, ALM P, ANDERSSON K-E: **Distribution and function of nitric oxide-containing nerves in canine corpus cavernosum and spongiosum.** *Acta Physiologica Scandinavia* (1995) **155**:445-455.

66. GOCMEN C, UCAR P, SINGIRIK E, DIKMEN A, BAYSAL F: **An *in vitro* study of nonadrenergic-noncholinergic activity on the cavernous tissue of mouse.** *Urol. Res.* (1997) **25**:269-275.

67. GONZALEZ-CADAVID NF, MAGEE T, VERNET D *et al.*: **Expression of penile neuronal nitric oxide synthase (PnNOS) in the rat and mouse penile nerves.** *J. Urol.* (1999) **161**:219.

68. STANARIUS A, UCKERT S, MACHTENS SA, STIEF CG, WOLF G, JONAS U: **Immunocytochemical distribution of endothelial nitric oxide synthase (eNOS) in human corpus cavernosum.** *J. Urol.* (1999) **161**:221.

69. BYRNE RR, HENRY GD, SZUKALA SA *et al.*: **The immuno-expression of endothelin, nitric oxide synthase and vascular endothelial growth factor in human corporal tissues from impotent men with disease-related variation.** *J. Urol.* (1999) **161**:220.

70. BLOCH W, KLOTZ T, SEDLACZEK P, ZUMBE J, ENGELMANN U, ADDICKS K: **Evidence for the involvement of endothelial nitric oxide synthase from smooth muscle cells in the erectile function of the human corpus cavernosum.** *Urol. Res.* (1998) **26**:129-135.

71. RAJASEKARAN M, MONDAL D, AGRAWAL K, CHEN IL, HELLSTROM W, SIKKA S: ***Ex vivo* expression of nitric oxide synthase isoforms (eNOS/iNOS) and calmodulin in human penile cavernosal cells.** *J. Urol.* (1998) **160**:2210-2215.

72. DAIL WG, BARBA V, LEYBA L, GALINDO R: **Neural and endothelial nitric oxide synthase activity in rat penile erectile tissue.** *Cell Tissue Res.* (1995) **282**:109-116.

73. GONZALEZ CM, BRANNIGAN RE, BERVIG T, MCKENNA KE, MCVARY KT: **Nitric oxide synthase I and III protein**

and gene expression in rat penes. *J. Urol.* (1999) **161**:220.

74. SULLIVAN ME, BELL CRW, DASHWOOD MR *et al.*: **Autoradiographic localization of nitric oxide synthase binding sites in normal and diabetic rat corpus cavernosum.** *Eur. Urol.* (1996) **30**:506-511.

75. TSUTSUI T, TAKEDA M, HATANO A, SUWA M, TAKAHASHI K: **Different distribution of caveolin-1 and caveolin-3 in the human corpus cavernosum: immunohistochemical study, in comparison with nitric oxide synthase nerve and low affinity growth factor receptors.** *J. Urol.* (1999) **161**:218.

76. ANDERSSON K-E, WAGNER G: **Physiology of erection.** *Physiol. Rev.* (1995) **75**(1):191-236.
• Excellent thorough review of penile physiology.

77. TAMURA M, KAGAWA S, KIMURA K, KAWANISHI Y, TSURUO Y, ISHIMURA K: **Coexistence of nitric oxide synthase, tyrosine hydroxylase and vasoactive intestinal polypeptide in human penile tissue - a triple histochemical and immunohistochemical study.** *J. Urol.* (1995) **153**:530-534.

78. BURNETT AL, TILLMAN SL, CHANG TSK *et al.*: **Immunohistochemical localization of nitric oxide synthase in the autonomic innervation of the human penis.** *J. Urol.* (1993) **150**:73-76.

79. HEDLUND P, ALM P, ANDERSSON K-E: **NO synthase in cholinergic nerves and NO-induced relaxation in the rat isolated corpus cavernosum.** *Br. J. Pharmacol.* (1999) **126**:349-360.

80. SAENZ DE TEJADA I, KIM N, LAGAN I, KRANE RJ, GOLDSTEIN I: **Regulation of adrenergic activity in penile corpus cavernosum.** *J. Urol.* (1989) **142**:1117-1121.

81. HOLMQUIST BF, HEDLUND H, ANDERSSON K-E: **Characterization of inhibitory neurotransmission in the isolated corpus cavernosum from rabbit and man.** *J. Physiol.* (1992) **449**:295-311.

82. CARTLEDGE J, EARDLEY I: **Viagra and beyond.** *Current Opinion in Central & Peripheral Nervous System Investigational Drugs* (1999) **1**:240-247.

83. TAHER A, MEYER M, STIEF CG, JONAS U, FORSSMANN WG: **Cyclic nucleotide phosphodiesterase in human cavernous smooth muscle.** *World J. Urol.* (1997) **15**:32-35.

84. KIELY EA, IGNOTUS P, WILLIAMS G: **Penile function following intracavernosal injection of vasoactive agents or saline.** *Br. J. Urol.* (1987) **59**:473-476.

85. KUKOVETZ WR, POCH G: **Inhibition of cyclic-3′,5′-nucleotide phosphodiesterase as a possible mode of action of papaverine and similarly acting drugs.** *Naunyn-Schmiederbergs Archives of Pharmacology* (1970) **267**:189-194.

86. BRADING AF, BURDYGA TV, SCRIPNYUK ZD: **The effects of papaverine on the electrical and mechanical activity of the guinea-pig ureter.** *J. Physiol.* (1983) **334**:79.

87. LOMAS GM, JAROW JP: **Risk factors for papaverine-induced priapism.** *J. Urol.* (1992) **147**:1280.

88. BOOLELL M, ALLEN MJ, BALLARD SA *et al.*: **Sildenafil: an orally-active Type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction.** *Int. J. Impot. Res.* (1996) **8**:47-52.
• First clinical report of the use of sildenafil.

89. BALLARD SA, TURNER LA, NAYLOR AM: **Sildenafil, a potent selective inhibitor of type-5 phosphodiesterase enhances nitric oxide dependent relaxation of rabbit corpus cavernosum.** *Br. J. Pharmacol.* (1996) **118**:153P.

90. JEREMY JY, BALLARD SA, NAYLOR AM, MILLER MAW, ANGELINI GD: **Effects of sildenafil, a type-5 cGMP phosphodiesterase inhibitor and papaverine on cyclic GMP and cyclic AMP levels in the rabbit corpus cavernosum *in vitro*.** *Br. J. Urol.* (1997) **79**:958-963.

91. MORELAND RB, GOLDSTEIN I, TRAISH A: **Sildenafil, a novel inhibitor of phosphodiesterase Type 5 in human corpus cavernosum smooth muscle cells.** *Life Sciences* (1998) **62**:PL309-318.

92. GOLDSTEIN I, LUE TF, PADMA-NATHAN H, ROSEN RC, STEERS WD, WICKER PA: **Oral sildenafil in the treatment of erectile dysfunction.** *N. Engl. J. Med.* (1998) **338**:1397-1404.
• Largest randomised trial of the use of sildenafil.

93. KASSIM AA, FABRY ME, NAGEL RL: **Acute priapism associated with the use of sildenafil in a patient with sickle cell trait.** *Blood* (2000) **95**:1878-1879.

94. KANE CJ, SUR RL: **Sildenafil citrate-associated priapism.** *Urology* (2000) **55**:950.

95. GIULIANO F, PORST H, PADMA-NATHAN H *et al.*: **Daily and on-demand IC351 treatment of erectile dysfunction.** *J. Urol.* (2000):894.

96. STARK S, SACHSE R, STARK A, LIEDL T, SCHAFHAUSER W, SCHROTT KM: **Erectile response on visual sexual stimulation after 20 mg or 40 mg BAY 38-9456 or placebo.** *Eur. Urol.* (2000) **37**(S2):4.

97. NOTO T, INOUE H, IKEO T, KIKKAWA K: **Potentiation of penile tumescence by T-1032, a new potent and specific phosphodiesterase Type V inhibitor, in dogs.** *J. Pharmacol. Exp. Ther.* (2000) **294**:870-875.

98. BIVALACQUA TJ, CHAMPION HC, RAJASEKARAN M *et al.*: **Potentiation of erectile response and cAMP accumulation by combination of prostaglandin E1 and rolipram, a selective inhibitor of the Type 4 phosphodiesterase (PDE4).** *J. Urol.* (1999) **162**:1848-1855.

99. RAJFER J, ARONSON WJ, BUSH PA, DOREY FJ, IGNARRO LJ: **Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission.** *N. Engl. J. Med.* (1992) **326**:90-94.

100. ZORGNIOTTI AW, LIZZA AF: **Effect of large doses of nitric oxide precursor L-arginine, on erectile function.** *Int.J. Impot. Res.* (1994) **6**:33-34.

101. CHEN J, WOLLMAN Y, CHERNICHOVSKY T, IAINA A, SOFER M, MATZKIN H: **Effect of oral administration of high dose nitric oxide donor L-arginine in men with organic erectile dysfunction: results of a double-blind,**

randomised, placebo-controlled study. *Br. J. Urol.* (1999) **83**:269-273.

102. CHAMPION HC, BIVALACQUA TJ, HYMAN AL, IGNARRO LJ, HELLSTROM WJG, KADOWITZ PJ: **Gene transfer of endothelial nitric oxide synthase to the penis augments erectile responses in the aged rat.** *Proc. Natl. Acad. Sci. USA* (1999) **96**:11648-11652.

Jon Cartledge†, Suks Minhas & Ian Eardley
†Author for correspondence
The Pyrah Department of Urology, St James's University Hospital, Beckett Street, Leeds, LS18 4AW, UK
Tel.: +44 0113 243 3144;
E-mail: j.Cartledge@ukgateway.net