Perspectives in Pharmacology

The Biochemical and Neurologic Basis for the Treatment of Male Erectile Dysfunction

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Male erectile dysfunction (MED) is defined as the “inability to achieve or maintain an (penile) erection adequate for sexual satisfaction” (National Institutes of Health Consensus Statement, 1993). Erectile dysfunction occurs in varying degrees in an estimated 20 to 30 million U.S. men and is associated with adverse effects on quality of life, particularly personal well being, family, and social interrelationships (Laumann et al., 1999; Johannes et al., 2000). The worldwide prevalence of MED has been estimated at over 152 million men, and the projections for 2025 suggest a prevalence of approximately 322 million with MED. Recent reports of quality of life data suggest that the importance of MED in contributing to other chronic health conditions such as depression has been largely underestimated (Laumann et al., 1999).

The treatment of MED has been revolutionized over the last decade from only surgical options (penile prostheses or revascularization) to intracavernosal and intraurethral administered agents [e.g., prostaglandin E1 (PGE1), papaverine, phentolamine] that paved the way to an effective oral therapy such as sildenafil. The clinical efficacy of oral agents such as sildenafil, apomorphine, phentolamine, IC351, and vardenafil represent the beginnings of noninvasive pharmacological treatment for MED.

Over the past 25 years, research on MED has focused on the mechanisms of corpus cavernosum smooth muscle relaxation, and this work has provided the basis for our current knowledge of the physiology of erection. On the other hand, the fields of experimental psychology and neuroscience have also unraveled critical information on the brain areas and the neuroanatomical connections regulating sexual behavior. In view of the latest developments in the clinical arena and the potential utility of several novel molecular targets for the pharmacological treatment of MED (Moreland et al., 2000), this review will summarize biochemical and neural mechanisms that affect corpus cavernosum smooth muscle tone and penile erection.

Epidemiology

Erectile dysfunction or impotence throughout most of the 20th century was considered predominantly as a psychological condition. The National Institutes of Health Consensus Development Panel on Impotence recognized that although it is difficult to separate psychogenic effects from organic disease, vasculogenic impotence accounts for about 75% of MED patients (National Institutes of Health Consensus Statement, 1993). In the literature before 1993, the term impotence is used and encompasses all forms of MED. Epidemiological studies suggest an association between impotence and increasing age and/or peripheral vascular disease (Laumann et al., 1999; Johannes et al., 2000). One recent study examined a random population of 1709 noninstitutional men aged 40 to 70 in the Boston area (Feldman et al., 1994; Johannes et al., 2000). The overall probability of some degree of sexual dysfunction was 52%. After adjusting for age, impotence was correlated with heart disease (39%), diabetes (28%), and hypertension (15%) as well as other vascular risk factors such as cigarette smoking. Treated heart disease (vasodilating drugs 36%), use of cardiac drugs (28%), and use of antihypertensive agents were also strongly associated. Correlations were also found with untreated medical conditions such as ulcers (18%), arthritis (15%), and allergies (12%). The follow-up study almost 9 years later allowed estimation of a risk of MED of about 26 cases per 1000 men annually, with correlation with vascular risk factors (heart disease, diabetes, hypertension).

ABBREVIATIONS: MED, male erectile dysfunction; PG, prostaglandin; ET, endothelin; ACh, acetylcholine; NOS, nitric-oxide synthase; NO, nitric oxide; CNS, central nervous system; NANC, nonadrenergic-noncholinergic; VIP, vasoactive intestinal peptide; CGRP, calcitonin gene-related peptide; PKG, cGMP-dependent protein kinase; MPOA, medial preoptic area; PVN, paraventricular nucleus; DA, dopamine; nPGI, nucleus paragigantocellularis; 5-HT, 5-hydroxytryptamine; TFMP, trifluoro-methylphenyl piperazine; mCPP, meta-chlorophenylpiperazine; 5-MeODT, 5-methoxy-N,N-dimethyl-tryptamine; TGF-β, transforming growth factor β; ICP, intracavernosal pressure; PDE, phosphodiesterase; EP, PGE receptor; 3-PPP, 3-(3-hydroxyphenyl)-N-(1-propyl)piperidine; M, muscarinic.
Physiology of Penile Erection

The tone of the corpus cavernosum smooth muscle is controlled by complex biochemical events coordinated at the level of the peripheral and central nervous system (Figs. 1 and 2). Sympathetic, parasympathetic autonomic, and somatic nerves control the tone of the corpus cavernosum smooth muscle and its vascular system via neuroanatomical connections that are an integral part of the innervation of the lower urinary tract (de Groat and Booth, 1993; Andersson and Wagner, 1995).

Peripheral Control of Penile Erection. The penis is composed of three bodies of erectile tissue: the corpus spongiosum, encompassing the urethra and terminating in the glans penis, and the two corpora cavernosa, which function as blood-filled capacitors, providing structure to the erect organ (de Groat and Booth, 1993; Andersson and Wagner, 1995). The corpus cavernosum is a unique vascular bed consisting of sinuses (the trabeculae) whose arterial blood supply arises from the resistance helicine arterioles, which in turn are fed from the deep penile cavernosal artery. The trabeculae are drained by the emissary venules that in turn communicate with the cavernosal veins. The trabeculae, while arterially fed, have measured blood PO2 of 20 to 40 mm Hg when the penis is in the flaccid state (Kim et al., 1993).

Ultimately, corpus cavernosum smooth muscle tone regulates penile flaccidity and erection (Fig. 1). During flaccidity, the helicine resistance arterioles are constricted, principally through α1-adrenergic mechanisms (Traish et al., 1999). Sympathetic efferents, via T11 through L2 including the pudendal nerve, supply a norepinephrine-induced tone in the corpus cavernosum, insuring flaccidity. Within the corpus cavernosum, α1a-, α1d-, α2a-, and α2c-adrenoceptors are expressed in the smooth muscle, while α1b- and α2d-adrenoceptors are expressed in the endothelium and/or nerves (Traish et al., 1999; Fig. 1). Endothelin (ET) synthesized both by the smooth muscle and endothelium may also play an autocrine/paracrine role in the maintenance of smooth muscle tone. Both types of endothelin receptors (ET A and ET B) are present in corpus cavernosum tissue (Andersson and Wagner, 1995). Other vasoconstrictors in this tissue include prostaglandin F2α (PGF2α) and thromboxane A2, synthesized both by smooth muscle and endothelium. Both PGF2α and thromboxane A2 potently constrict corpus cavernosum strips in organ baths and may be elevated in vascular complications such as diabetes and hypercholesterolemia. Functional M3 and M4 muscarinic acetylcholine (ACh) receptors have been demonstrated in human corpus cavernosum smooth muscle and cultured smooth muscle cells (Nehra et al., 1999). As these receptors are coupled to G i proteins, they would be expected to result in corpus cavernosum smooth muscle contraction. However, carbachol relaxes phenylephrine-precontracted human corpus cavernosum strips in organ baths, and this process is thought to proceed through M3 receptors on the endothelium. In this case, ACh increases intracellular calcium via M3 receptors, which in turn activate endothelial nitric-oxide synthase (NOS) (Andersson and Wagner, 1995). The endothelial synthesis of nitric oxide (NO) acts on the underlying smooth muscle in an autocrine fashion to potentiate corpus cavernosum smooth muscle relaxation.

Penile erection is the end result of smooth muscle relaxation, and this relaxation can be initiated by sensory stimulation that activates central nervous system (CNS) pathways (de Groat and Booth, 1993; Andersson and Wagner, 1995). These processes activate peripheral nerves innervating the penis, which include cholinergic, nonadrenergic-noncholinergic (NANC; e.g., NO), vasoactive intestinal peptide (VIP)-, and potentially calcitonin gene-related peptide (CGRP)-con-
taining nerves entering the pelvic plexus from S2–S4. NO from NANC nerves mediates the dilation of the helicine arterioles as well as the trabecular smooth muscle. The influx of arterial blood is associated with a rise in blood PO$_2$ (90–100 mm Hg), and this increase in oxygen tension further activates NOS as well as prostaglandin G/H synthase, which both utilize molecular oxygen as a substrate (Kim et al., 1993). Shear stress and muscarinic ACh receptors on the corpus cavernosum endothelium increase intracellular calcium, activating the endothelial NOS and stimulating the production of NO. NO diffuses into the smooth muscle, further enhancing relaxation (Fig. 1).

As the corpus cavernosum sinuses relax and fill with blood, intracavernosal pressure and volume increase. Veno-occlusion develops through stretching and compressive forces by expandable corpus cavernosum tissue on subtunical venules, and the penis becomes a blood-filled capacitor (Andersson and Wagner, 1995). VIP- and CGRP-mediated pathways proceed through cAMP-dependent pathways concomitant with the NANC nerve-mediated pathways and also contribute to corpus cavernosum smooth muscle relaxation. VIP and CGRP receptors remain to be fully characterized in this tissue. PGE is synthesized by the corpus cavernosum endothelial and smooth muscle cells and binds to specific PGE (EP) receptors on the smooth muscle, which can also increase intracellular cAMP levels and potentiate smooth muscle relaxation. PGE can bind to four different pharmacologic classes of receptor, and all four are expressed in the penis (Moreland et al., 2000). Both EP$_2$ and EP$_4$ are coupled to Gs and are probably responsible for the increased cAMP synthesis observed in tissues and in cultured smooth muscle cells when these are treated with exogenous PGE$_1$ (Narumiya et al., 1999). Research continues to elucidate other receptors and potential pathways for mediating corpus cavernosum smooth muscle tone.

While the pathways involved in smooth muscle myosin phosphorylation and dephosphorylation that are fundamental for corpus cavernosum smooth muscle tone remain to be elucidated, it is clear that intracellular calcium as well as ion channels play a key role. K$_{ATP}$, Maxi K, L-type calcium channels, and sodium potassium ATPase have all been identified in corpus cavernosum smooth muscle. K$_{ATP}$ channel openers like cromakalin can relax corpus cavernosum strips in organ baths, and nicorandil can relax corpus cavernosum albeit by a dual mechanism that includes K$_{ATP}$ channel opening and guanylate cyclase activation (Hsieh et al., 2000). Transfecting Maxi K channels into aging rat corpus cavernosum has been shown to improve erectile function (Christ et al., 1998).

The relative importance of the cAMP and cGMP pathways is still unclear, but recent studies have observed that mice lacking cGMP-dependent protein kinase I (PKG-I) fail to reproduce (Hedlund et al., 2000). Corpus cavernosum strips from these mice do not relax in organ baths upon activation of the NO-cGMP cascade. However, forskolin, which directly activates adenylate cyclase and the cAMP pathway, relaxes phenylephrine-precontracted tissues, although to a significantly lesser degree than in the wild-type mice. As the cAMP system remained intact, these data demonstrate that the cAMP pathways alone cannot compensate for the cGMP deficiencies in vivo. Alternatively, PKG (cAMP/cGMP cross-talk) may be essential in some portion of the adenylate cyclase signaling pathway in these cells.

Central Mechanisms Controlling Penile Erection.

The neural pathways involved in penile erection are just beginning to be integrated into a unified body of knowledge as the role of specific CNS pathways is corroborated by experimental findings from independent laboratories. The different areas in the brain and the neuroanatomical connections presently known to regulate penile erection are shown diagrammatically in Fig. 2. External information can stimulate sexual activity via the different sensory (visual, olfactory, tactile, and auditory) pathways that, with the exception of the olfactory system, reach the corresponding cortical areas and then project to polymodal cortical association areas. The piriform and entorhinal cortices have extensive neuronal connections with limbic structures like the amygdala (de Groot and Booth, 1993). Lesions of the medial amygdala significantly impaired copulatory behavior in rats, while bilateral lesions of the temporal lobes including the amygdala induced a syndrome of hypersexuality and frequent penile erections in monkeys known as the Kluver-Bucy syndrome (Kluver and Bucy, 1938). Similar behavioral changes in sexual behavior have also been observed in humans, suggesting that the amygdala plays an important role regulating male sexual activity besides its participation in learning, memory, and the control of emotional behavior (Brioni, 1993).

Connections between the amygdala and midbrain structures like the periaqueductal gray and the hypothalamus have been described in several species. Stimulation and electrolytic lesions of the periaqueductal gray modulate sexual activity as well as the selective lesioning or stimulation of the medial preoptic area (MPOA) and the paraventricular nucleus (PVN) of the hypothalamus in the rat (McKenna, 1998).

![Fig. 2. Neuroanatomical connections and putative neurotransmitters in the central nervous system involved in penile erection.](https://jpet.aspetjournals.org/)

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The MPOA and PVN nuclei play a critical role in sexual behavior as bilateral lesions of these areas completely eliminate male sexual behavior; this effect is also observed after lesion of the medial forebrain bundle, the major efferent pathway of the hypothalamic centers to the midbrain. Although the primary efferents of the MPOA project to the midbrain area via the medial forebrain bundle, the MPOA also sends projections to the PVN.

The zona incerta is a diencephalic structure that represents the rostral extension of the reticular formation. The incerto-hypothalamic dopaminergic pathway (originally described as the A13 group) arises from the medial part of the zona incerta and innervates the amygdala, the PVN, and the MPOA nuclei. Substantial preclinical evidence demonstrates that central dopamine (DA) plays a role in penile erection in animals. Systemic administration of DA receptor agonists like apomorphine, quinpirole, lisuride, quinelorane, and 3-PPP facilitate penile erection in rats and rabbits (Bitran and Hull, 1987), an effect blocked by haloperidol, a central DA antagonist. As the eectogenic effect cannot be blocked by domperidone, a peripheral DA antagonist, it is believed that the proerectile effect of DA agonists is centrally mediated (Morales et al., 1995). Systemic injections of apomorphine increase intracavernosal pressure in awake rats, and this effect can be blocked by bilateral transection of the pudendal or cavernous nerves (Andersson et al., 1999). Intracerebroventricular injections of apomorphine as well as localized injections in the hypothalamus facilitate penile erections, while injections in the striatum and the nucleus accumbens do not reach similar efficacy (Andersson et al., 1999). Direct injections of apomorphine into the PVN of the hypothalamus significantly increase intracavernosal pressure in rats (Chen et al., 1999). These data suggest that the incerto-hypothalamic dopaminergic projection that innervates both the MPOA and PVN of the hypothalamus plays an important role regulating male sexual activity. The participation of other DA pathways cannot be completely ruled out as the mesolimbic and mesocortical DA systems play significant roles in motivation, emotional, and reward mechanisms (Bitran and Hull, 1987; Melis and Argiolas, 1995).

DA agonists increase NO in the PVN of the hypothalamus. This suggests a role for the NO-cGMP system in the CNS besides its action at the level of the cavernosal smooth muscle (Andersson et al., 1999; Sato et al., 2000). A potential site of action of NO is the PVN in the hypothalamus as administration of nitroglycerin or l-arginine in the PVN induces penile erections, and injections of Nω-nitro-l-arginine methyl ester (l-NAME) in the PVN block apomorphine- and oxytocin-induced erections in rats (Argiolas and Melis, 1995). The central role for NO is further emphasized in studies in which the phosphodiesterase 5-selective inhibitor sildenafil was injected intrathecally in a rat animal model (Sato et al., 2000). A significant increase in MPOA-stimulated erections monitored by intracavernous pressure increases was noted in the presence of sildenafil. It is unknown whether sildenafil citrate crosses the blood-brain barrier in humans and exerts a CNS effect on penile erection. However, in clinical trials, sildenafil did not have a significant effect on libido or desire, both CNS measures (Goldstein et al., 1998). These specific actions in the PVN suggest that NO activity in the PVN is a common mediator of the effects of several central neurotransmitters on penile function (Argiolas and Melis, 1995). The central NO-cGMP system can regulate penile erection in view of the experimental evidence in rats and could represent a potential target for the development of new therapeutic agents for the treatment of MED.

The proerectile effect of DA agonists may also be related to their ability to release oxytocin. The doses of apomorphine that induce penile erection also increase plasma levels of oxytocin, and oxytocin antagonists can block apomorphine-induced erections. Haloperidol blocks apomorphine-induced erections, but it is unable to block oxytocin-induced erections, indicating that oxytocin release is a downstream neurochemical event related to the proerectile action of apomorphine (Carter, 1992).

Oxytocinergic neurons from the parvocellular division of the PVN specifically project to the spinal cord, a paraventriculo-spinal projection to thoracolumbar and lumbosacral spinal neurons that control penile erection in rats (Veronneau-Longueville et al., 1999). Oxytocin induces a significant facilitatory effect on penile erection when injected in the PVN. The proerectile effect of oxytocin can be blocked by specific oxytocin antagonists and by Ω-conotoxin, indicating that this effect is mediated by oxytocin receptors linked to voltage-dependent calcium channels (Argiolas and Melis, 1995).

Limbic structures send projections to areas of the pons-medulla that are critical for regulation of male sexual and urinary function. The MPOA provides a dense and selective innervation to discrete regions in the pons, namely Barrington’s nucleus and surrounding areas of the locus coeruleus. Barrington’s nucleus is believed to be a regulator of pelvic visceral function as it projects to sacral parasympathetic nucleus known to regulate the micturition reflex and gastrointestinal and genital function (Valentino et al., 1999). Terminals from neurons located in Barrington’s nucleus form excitatory synaptic apices with preganglionic parasympathetic neurons, indicating that it exerts a direct excitatory effect on pelvic function probably by releasing corticotrophin-releasing factor. Spinal and brainstem neurons that innervate penile muscles have been identified by trans-neuronal tracing techniques. Pseudorabies virus injection in penile muscles labeled spinal interneurons, spinal sympathetic, and parasympathetic preganglionic neurons, as well as neurons in the raphe, periaqueductal gray area, locus coeruleus, and Barrington’s nucleus (McKenna, 1998). Electrical stimulation of the Barrington’s nucleus slightly increased intracavernosal pressure in decerebrate rats, while stimulation of areas ventrolateral and caudal to the Barrington’s nucleus significantly increased intracavernosal pressure, demonstrating their role in male sexual function (Moreland et al., 2000). The sacral projections of the Barrington’s nucleus, its connection to the locus coeruleus, and the afferent projections coming from the periaqueductal gray, MPOA, lateral hypothalamus, and cortex, allow it to integrate lumbosacral parasympathetic activity and limbic/forebrain activity to coordinate pelvic visceral information and behavioral responses (Valentino et al., 1999).

Anatomical studies have also identified an important source of 5-HT neurons related to sexual function, as injection of pseudorabies virus into penile tissue consistently labeled neurons in the nucleus paragigantocellularis (nPGi). The projection from the nPGi to the sacral spinal cord is mainly serotonergic, and lesions of the nPGi facilitate pe-
nile reflexes in rats (McKenna, 1998). The effect of serotoninergic ligands on male sexual behavior has been extensively investigated due to the availability of a large number of agonists and antagonists. In vivo pharmacological studies showed that MK-212, TFMPP, mCPP, and Ro60-0175 facilitate penile erection in rats, while PAPP, 5-MeOMDT, ORG-6997, and BW723C86 are inactive (Moreland et al., 2000). Molecular biological and biochemical efforts have allowed the present classification of 5-HT receptors in seven families (5-HT1–7) comprising a total of 17 receptor subtypes (Barnes and Sharp, 1999). The available information suggests that 5-HT2C receptors are related to the facilitatory effect of 5-HT and that 5-HT1A receptors exert inhibitory effects on male sexual behavior. A detailed understanding of the role of the 5-HT subtypes on penile erection could be obtained after further pharmacological evaluation with available subtype-selective agents.

The proerectile effects of yohimbine (α2-adrenoceptor antagonist) in humans indicate that central noradrenergic systems could play a role in male sexual behavior. However, the efficacy of yohimbine in humans is modest, and it may be related to arousal or motivational aspects of sexual behavior (Morales et al., 1995). Recent data on the proerectile effect of phentolamine in phase III trials in humans has renewed interest in this area (Wyllie and Andersson, 1999). It is generally assumed that the proerectile effect of phentolamine is due to the blockade of α-adrenoceptors in the corpus cavernosum (Traish et al., 1999), but the blockade of adrenoceptors located in spinal or brain sites cannot be excluded. A recent report on the lack of effect of the adrenergic antagonist Ro70-004/003 (Choppin et al., 2000) continues to demonstrate that α-adrenoceptor blockade alone might not be useful for the treatment of MED.

The participation of other central neurotransmitters/neuromodulators and hormones (ACh, glutamate, γ-aminobutyric acid, adrenocorticotropic hormone, melanocyte-stimulating hormone, opioids, prolactin) has also been documented in relation to male sexual behavior and has been the subject of several reviews (Dornan and Malsbury, 1989; de Groat and Booth, 1993; Andersson and Wagner, 1995). Male sexual function is a complex behavioral phenomenon that is unique to each animal species, and for that reason extrapolations from animal (rat, rabbit, or monkey) to human sexual function should be made with care. Penile erection is part of a complex behavioral repertoire related to physiological and psychological aspects like motivation, reward, interaction with a receptive female, and ejaculation. A detailed knowledge on the central neurotransmitter systems involved in the control of sexual function will certainly be important for the discovery of novel pharmacological agents for the treatment of MED.

Pathophysiology of Male Erectile Dysfunction

MED can be classified into four types: 1) psychogenic, 2) vasculogenic or organic, 3) neurologic, and 4) endocrinologic. MED can also result as a side effect of pharmacological treatments such as antihypertensive medications (β-adrenoceptor blockers), serotonin reuptake inhibitors, diuretics, and cardiac medications (Meinhardt et al., 1997).

There are currently two hypotheses of pathophysiology: one proposes a structural basis of MED, while the other focuses on metabolic imbalances in the corpus cavernosum. The penis is comprised of soft tissue and functions as a blood-filled capacitor of sufficient rigidity during erection for vaginal penetration (Nehra et al., 1999). The two bodies of erectile tissue, the corpora cavernosa, that are integral to this function are composed of a specialized vascular bed, which has a high content of connective tissue (48–55%). The corpus cavernosum smooth muscle cells also synthesize connective tissue that contributes to the structural integrity of the corpora, and a functional corpus cavernosum smooth muscle/connective tissue ratio is necessary for veno-occlusion (Moreland, 1998). The implications of this finding are that regardless of the amount of corpus cavernosum smooth muscle relaxation, veno-occlusion cannot occur in some patients due to higher content of connective tissue and an inability to occlude the draining venules.

One area of active research in MED is to identify vasoactive factors, cytokines, autacoids, and/or neurotransmitters that may play a role in maintaining the connective tissue/smooth muscle balance (Moreland, 1998). Among the potential candidates are transforming growth factor β1 (TGF-β1) and prostaglandin E, both of which are synthesized by the corpus cavernosum smooth muscle cells. These vasoactive substances are regulated by oxygen tension; TGF-β1 is induced under lower oxygen tension conditions consistent with flaccidity, while PGE is synthesized under conditions consistent with oxygen tensions during erection. In human corpus cavernosum smooth muscle cells in culture, TGF-β1 can induce a 2.5- to 4-fold increase in collagen synthesis in these cells, and this synthesis can be repressed by a single dose of PGE1. While these are in vitro observations, it is interesting to note that nocturnal penile tumescence may provide a daily oxygenation of the corpus cavernosum regardless of sexual activity that may help to maintain a functional corpus cavernosum smooth muscle/connective tissue balance (Moreland, 1998). While research has yet to supply an answer to a number of the questions involved, strategies to alter the corpus cavernosum structure could be a future means to treat MED.

An alternate hypothesis regarding the pathophysiology of MED is attributed to a metabolic imbalance between contractile and relaxatory factors in the corpus cavernosum (dysfunctional antagonism; Melman and Gingell, 1999). Under normal physiological conditions in the penis, contractile factors (norepinephrine, ET, and contractile prostanoids) are in balance with relaxatory factors (NO, VIP) such that when the contraction of the corpus cavernosum diminishes and relaxatory factors are present, erection ensues. In the case of dysfunctional antagonism, contractile factors predominate, are overexpressed or relaxatory factors are inhibited, such that the trabecular smooth muscle remains contracted and the penis remains flaccid. However, in most cases of vasculogenic MED, a decrease in NO production and release probably plays some role in the dysfunction. In actual practice, the pathophysiology of erectile dysfunction probably has both structural and metabolic components.

Experimental Approaches to Study MED

In Vitro Models. As penile erection involves relaxation of corporal vascular and smooth muscle during sexual stimulation, the application of in vitro models of isolated corpus
cavernosal tissue has significantly enhanced our understanding of the biochemical events at the cellular level (Andersson and Wagner, 1995). From a scientific perspective, these models allow dissection of the various neurotransmitters and vasoactive factors involved in this process and the signal transduction pathways therein.

There are two major in vitro models currently in use: muscle strips in organ baths and cell cultures derived from the corpus cavernosum. Central nervous system experiments involving brain slices have yet to be used with great utility in this area of research. Sources of tissues for experimentation have relied on human surgical biopsies of corpus cavernosum obtained at the time of penile prosthesis insertion in men with MED or from penile cancer patients undergoing partial penectomy. The restricted availability of human penile erectile tissues has lead to the use of cavernosal tissues isolated from rabbit as a major in vitro model both for screening and for detailed analysis of mechanisms previously demonstrated to exist in human tissue. Similar information has been obtained by using tissues from mouse, rat, dog, horse, and pig (Andersson and Wagner, 1995; Giuliano et al., 1999). The advantages of this method include ease in preparation, minimal equipment requirements, and the fact that reproducible concentration-effect curves can be obtained when studying either contractile or relaxant agents. The response to pharmacological stimulation by adding contracting agents such as phenylephrine, potassium chloride, or other contractile factors is the most common method used to contract the corpus cavernosal tissues in vitro to assess potency and efficacy of relaxing agents. Smooth muscle strip contractions in organ bath preparations can also be achieved by electric field stimulation similar to that used to study neural responses (Kim et al., 1993).

During the last decade, primary cultures of cavernosal cells from human or animals have provided useful insights into factors that can modulate the intracellular events. These studies have included cultures of human corpus cavernosum endothelial as well as smooth muscle cells (Moreland et al., 2000). Such experiments have been helpful in defining which receptors are localized in each cell type within the corpus cavernosum and in understanding the association to specific signal transduction mechanisms. As the degree of smooth muscle tone is mainly controlled by the intracellular Ca\(^{2+}\) concentration (Horowitz et al., 1996), any event that limits Ca\(^{2+}\) entry to the cell or release of Ca\(^{2+}\) from intracellular storage will ultimately have a significant impact on corporal smooth muscle tone (Andersson and Wagner, 1995; Stief et al., 1997). Two main intracellular messenger molecules, cAMP and cGMP, modulate the continuous transmembrane Ca\(^{2+}\) flux through voltage-dependent calcium channels that are critical to the sustained contraction of corporal smooth muscle. Studies in tissue strips and primary cell cultures have provided the available information on intracellular events described in Fig. 1.

**In Vivo Animal Models.** Intracavernosal pressure (ICP) changes in animal models as an index of penile erection have greatly enhanced our understanding of basic erectogenic pathways and systems. As discussed above, ICP increases as the corpus cavernosum relaxes and fills with blood, and the plateau of ICP is indicative of successful veno-occlusion and functional erection. Some researchers prefer ICP models for studies of penile erection to in vitro organ bath chamber experiments. Both approaches have merit in dissecting various aspects of male erectile physiology. Advantages of the ICP model aside from an intact animal preparation include the ability to perform continuous monitoring of ICP, duration of erection, and concomitant measurement of arterial pressure. A combination of intracavernosal drug administration and selective nerve stimulation or ablation has greatly increased understanding of the neurophysiology of erection (Andersson and Wagner, 1995; Christ et al., 1998; Sato et al., 2000). Many physiological studies on penile erection are carried out in larger animals, including monkeys, dogs, and cats (Giuliano et al., 1999). The large volume of tissues and prominent peripheral nervous and blood vessel supplies to the penis in these animals provide convenient access for pharmacological, neurological, and hemodynamic evaluations. The rabbit model has also been used for studying the erectile response to the intracavernosal injection of vasoactive drugs. The important differences among species should be considered for every specific study.

The rat has been a primary animal model for studying penile erection in vivo. Mating tests have been accepted as a standard, and the parameters of mating tests are well established (cup, flips, number of intromissions, latency to mounting, and latency to ejaculation) (Bitran and Hull, 1987; de Groat and Booth, 1993; Pfau, 1999). These observational tests document a range of interlinked behaviors among which erection is one component. The female rat, ovarioectomized and primed with estradiol before the copulatory testing, responds to the mounting from the male rat with the lordosis response (a primary behavioral component of female sexual behaviors): dorsoflexion of spine and deflection of the tail to one side, allowing vaginal access to the male (Bitran and Hull, 1987; Pfau, 1999).

Since subcutaneous injection of apomorphine causes an observable erectile response in nearly all normal rats, apomorphine administration can be used as a standard challenge to produce a quantifiable erectile response. This simple model can also be used to determine augmentation and synergy with different agents that when administered alone do not directly induce erection (Morales et al., 1995; Giuliano et al., 1999). An example of such a study is the recent report that apomorphine augments the effects of sildenafil on ICP following nerve stimulation in a rat model (Andersson et al., 1999). The model can also be applied to monitor ICP or oxygen tension-mediated changes if one uses anesthetized rats.

Erectile dysfunction is associated with vascular risk factors (Feldman et al., 1994; Laumann et al., 1999; Johannes et al., 2000). Unfortunately, by the time a patient presents with MED, the endpoints of disease may be well on their way to being obtained. Animal models of disease offer an opportunity to understand the mechanisms of pathophysiology with the progression of the disease. A variety of potential animal models have been described based on associated risk factors, especially diabetes, aging, atherosclerosis, and other vascular disorders (Giuliano et al., 1999). Erectile dysfunction is a common and devastating consequence of diabetes in adult men. Studies in animal models of diabetes have revealed pathological changes in the penile arteries, morphological alterations of autonomic nerves, and a depletion of neurotransmitters within the corpus cavernosum. Several strategies have been developed in chemical-induced diabetes (hy-
perglycemia-dependent) in rats or rabbits to analyze neural, vascular, and biochemical changes. Both CNS (hypothalamic MPOA) and peripheral (pelvic nerve) nerve stimulation (Giuliano et al., 1999) have described streptozotocin-induced diabetes in rats as a neuropathy model to assess penile erection. Rabbits fed with alloxan for 8 weeks to destroy pancreatic islet cells have also been established as a MED chronic diabetic model that mimics the impaired NOS response as well as induction of constrictor prostanoids. Hypercholesterolemia is a cardiovascular risk factor and also a predictor of MED in both humans and animal models (Feldman et al., 1994; Johannes et al., 2000). Rabbits fed with a diet enriched with cholesterol for 16 weeks are able to produce functional hypercholesterolemia, and the structural and morphometric changes within the cavernosal tissues have been characterized (Giuliano et al., 1999). Although this type of model can be used in the identification of agents that affect the underlying disease progression rather than of agents that produce acute symptomatic relief, it is limited by the extreme conditions to induce the model. Another important aspect of drug discovery is the design of clinical trials. A review of recent clinical trials for MED and tools for assessing efficacy have been recently published (Moreland et al., 2000).

**Advances in the Treatment of MED**

A variety of drug targets have been proposed for the potential treatment of MED. The large number and diversity of targets is indicative of the significant interest in the pharmaceutical arena to identify novel agents for MED, as it is still an unmet medical need for a substantial portion of patients. In view of the recent success of sildenafil, a major area of activity is in the development of PDE5 inhibitors. There is also interest in several other molecular targets at the smooth muscle level as well as in the CNS. Drugs used presently in clinical practice are shown in Table 1 and Fig. 1. Peripheral treatment of erectile dysfunction focuses on enhancing corpus cavernous smooth muscle relaxation as described below.

![Table 1](https://www.aspetjournals.org/jpet/)

**Agents That Increase cAMP Synthesis.** The finding that erection proceeds through mechanisms that increase intracellular levels of cyclic nucleotides (cAMP and cGMP) have allowed the development of several classes of therapeutic agents. The first class of agents that increases intracellular cAMP synthesis works either via specific cell surface receptors, which are then coupled to adenylate cyclase, or drugs that activate adenylate cyclase directly. Prostaglandin E₁ binds to specific EP receptors on the corpus cavernosum smooth muscle cells, elevating intracellular cAMP levels by coupling through G<sub>a</sub> protein mechanism and activation of adenylate cyclase (Narumiya et al., 1999). This increase in cAMP activates a signal transduction cascade whose ultimate result is phosphorylation/dephosphorylation events with the actin-myosin system leading to smooth muscle relaxation (Fig. 1).

**PGE<sub>1</sub>** administered via intracorporal injection was the first therapeutic agent approved by the FDA to treat MED (Nehra et al., 1999; Spahn et al., 1999). As an injectable agent, PGE<sub>1</sub> is effective alone as well as in combination with other agents. Unlike papaverine, and papaverine in combination with phentolamine, PGE<sub>1</sub> injection is associated with a much lower incidence of corpus cavernosal fibrosis (Spahn et al., 1999). Formulations of PGE<sub>1</sub> with α-cyclodextrin designed to enhance its solubility and delivery have been reported (Spahn et al., 1999). Undue side effects of the injection of PGE<sub>1</sub> include penile pain (approximately 25% of patients), an effect probably related to the lack of selectivity of PGE<sub>1</sub> for the four EP receptor subtypes (Narumiya et al., 1999). Pain associated with PGE<sub>1</sub> administration may be due to cross-reactivity with EP<sub>1</sub> receptors in the corpus cavernosum. Another side effect can be prolonged erections (observed in about 4% of patients), which can result in veno-occlusive priapism (Spahn et al., 1999). On the other hand, PGE<sub>1</sub> can have a prophylactic effect on the recovery of erectile function following surgery. Radical retropubic prostatectomy, even when performed by nerve-sparing techniques, often results in veno-occlusive priapism (Spahn et al., 1999). On the other hand, PGE<sub>1</sub> can have a prophylactic effect on the recovery of erectile function following surgery. Radical retropubic prostatectomy, even when performed by nerve-sparing techniques, often results in either a temporary or permanent loss of erectile function. A recent prospective study of nerve-sparing radical prosta-
tectomy patients found that patients treated with postoperative, prophylactic intracavernosal PGE1 injections were more likely to recover erectile function than those on placebo (Nehra et al., 1999).

Intraurethral delivery of PGE1 has been proposed to mediate erection by drug entry via the urethra and into the draining cavernosal venules and the corpus cavernosum by retrograde flow (Nehra et al., 1999). Recently, this therapy was improved by the inclusion of a restriction band applied to the base of the penis before insertion of the suppository. The dosages for PGE1 range from 25 to 100 times that for corporal injection. While this means of drug delivery alleviates some of the unpleasantness of corporal injection, recent studies suggest that efficacy is low. This may reflect clinical trial selection of MED patients with minimal dysfunction followed by marketing to a wider range of MED etiologies or poor clinical trial endpoints. A recent development in intraurethral therapy has been the combination of the α-antagonist prazosin and PGE1 (Nehra et al., 1999).

VIP also increases intracorporeal smooth muscle cAMP synthesis by coupling with specific VIP receptors. While VIP may be one of the neurotransmitters involved in mediating erection, intracorporal injection of VIP alone does not result in erection (Andersson and Wagner, 1995). Injectable VIP/phenolamine combinations are in clinical trials in the United Kingdom. Forskolin, a plant diterpene from Coleus forskohlii, directly activates adenylate cyclase by a mechanism independent of G-protein coupling. Forskolin has been shown to elevate cAMP levels in human corpus cavernosum smooth muscle cells in culture and in particular can augment the effects of PGE. This property may result in an agent that is useful in multidrug formulations to improve efficacy (Nehra et al., 1999).

α-Adrenoceptor Antagonists. Sympathetic α-adrenoreceptors are thought to maintain the flaccidity of the corpus cavernosum (Andersson and Wagner, 1995; Traish et al., 1999), and blockade of these receptors by α1, α2-, or mixed α-adrenoceptor antagonists have been used to treat MED. In contrast to the general view that α-adrenoceptor antagonists act only at the level of the smooth muscle, these agents can act either in the CNS or peripherally pre- and postsynaptically.

Phentolamine has been administered intracavernosally as a single medication, but it is most effective in combination therapy (Spanhn et al., 1999). Oral phentolamine has undergone clinical trials in Europe and in the United States (Wyllie and Andersson, 1999). A recent study to determine whether the oral α-adrenoceptor antagonist doxazosin used to treat benign prostatic hypertrophy was effective in enhancing the effects of intracavernosal therapy found limited efficacy. However, it is interesting to note that in a study of the treatment of mild hypertension, patients taking doxazosin reported a lower incidence of erectile dysfunction. Similarly, tamsulosin has been reported to improve sexual function in benign prostatic hypertrophy patients (Moreland et al., 2000). Chlorpromazine, delequamine, moxisylyte, prazosin, and yohimbine act on α1- or α2-adrenoceptors, and in some cases both α-adrenoceptors in a combination of CNS and peripheral effects (Morales et al., 1995; Wyllie and Andersson, 1999). Trazodone has been shown to induce erection when injected intracavernosally. It has been further demonstrated that these effects are mediated by a dual mechanism including α-adrenoceptor antagonism and serotoninergic activity. Oral trazodone has been used to treat psychogenic erectile dysfunction (Nehra et al., 1999). Another oral agent for the treatment of psychogenic impotence is yohimbine, an α2-adrenoceptor antagonist.

It has recently been reported that the α1-adrenoceptor antagonist Ro70-0004/003 did not improve erectile function in a clinical study including 24 men (Choppin et al., 2000). The lack of effect of this adrenoceptor antagonist continues to demonstrate that orally administered α-antagonists might not be useful for the treatment of MED despite the positive data generated with oral phenolamine. The approval of phentolamine (Vasomax, Zonagen, Woodlands, TX) has recently been delayed by the FDA due to abnormal proliferation of brown fat tissue in rats, and this agent may not reach the market in the next 2 years.

Agents That Increase cGMP Synthesis. The discovery that NO is one of the major effectors in penile smooth muscle relaxation and erectile function has led to the development of two classes of agents: NO donors and agents that elevate and/or potentiate cGMP levels (PDE inhibitors). As discussed above, NO is synthesized by neural NOS in the NANC nerve terminals as well as by the corpus cavernosum endothelial cells (endothelial NOS) in response to shear stress, acetyl-choline, or bradykinin (Andersson and Wagner, 1995). Examples of drugs that work as NO donors include nitroglycerin, minoxidil, and sodium nitroprusside. However, NO donors themselves can activate guanylate cyclase not only in corpus cavernosum but also in other tissues because of the ubiquitous distribution of guanylate cyclase. Recently, the use of NO donors attached by nitrosylation either to α-receptor antagonists or to PDE inhibitors has been investigated. Attached NO was shown to significantly improve the therapeutic efficacy of both compound classes, increasing intracavernosal pressure and the duration of the erection. The development of these compounds, while promising, is still at the preclinical stage (Moreland et al., 2000).

PDE Inhibitors. PDEs are enzymes that hydrolyze cAMP and cGMP to their respective monophosphates to terminate signal transduction by these second messengers within the corpus cavernosum. To date, of the 11 known PDEs, types 2, 3, 4, and 5 have been identified in the corpus cavernosum (Stief et al., 1997; Corbin and Francis, 1999). PDE5 is the major cGMP hydrolytic activity in the corpus cavernosum smooth muscle cell (Corbin and Francis, 1999). Sildenafil is a selective PDE5 inhibitor that has been shown to be a safe and effective oral treatment for MED (Goldstein et al., 1998; Cheitlin et al., 1999). The mechanism of action of sildenafil requires an intact NO response as it blocks the hydrolysis of cGMP induced by NO as well as constitutive synthesis of cGMP in the cells. This may explain why sexual arousal is necessary for the effectiveness of sildenafil in men. Sildenafil is a potent competitive inhibitor of PDE5 (IC50 = 3.5 nM) and is selective over PDE1 to -4 (80- to 19,000-fold) and retinal PDE6 (10-fold). Sildenafil enhanced cGMP accumulation driven with NO in the corpus cavernosum of rabbits without affecting cAMP accumulation. More importantly, in the absence of NO release, sildenafil had no functional effect on the human and rabbit isolated corpus cavernosum but potentiated the relaxant effects of NO on these tissues (Moreland et al., 2000). In the anesthetized dog, sildenafil enhanced the increase in intracavernosal pressure induced by electrical
stimulation of the pelvic nerve or intracavernosal injection of sodium nitroprusside without effects on blood pressure. Consistent with its mode of action, sildenafil potentiated the vasorelaxant effects of glyceryl trinitrate on rabbit isolated aortic rings (Corbin and Francis, 1999).

The major cellular receptor for cGMP in causing vascular smooth muscle relaxation is PKG. PKG is thought to cause relaxation of the smooth muscle through lowering of cellular Ca$^{2+}$, which may involve phosphorylation of inositol triphosphate receptor, Ca$^{2+}$-ATPase, Ca$^{2+}$-channels, or other proteins (Andersson and Wagner, 1995; Stief et al., 1997). After cessation of erotic stimuli, NO release from the parasympathetic nerves of the penis declines, and the cGMP level in the smooth muscle cells falls because of a decrease in synthesis coupled with the ongoing degradation of cGMP by PDEs.

Papaverine was the first drug reported for intracavernosal injection (Spahn et al., 1999). It is thought that papaverine exerts part of its action as a nonselective PDE inhibitor. As such it would be expected to increase both cAMP and cGMP levels within the corpus cavernosum smooth muscle cell. Used now primarily in combination with other agents, the complications of papaverine include potential liver damage and the tendency to develop penile fibrosis. Although there are no cAMP PDE inhibitors available for the treatment of MED, the recent report of both type 3 and type 4 PDE in human corpus cavernosum (Stief et al., 1997; Nehra et al., 1999) leads to the possible use of drugs such as milrinone (type 3 PDE selective) or rolipram (type 4 PDE selective) in MED patients. However, the importance of cAMP for physiological functions in the heart and other tissues may preclude such an approach.

**ET Receptor Antagonists.** It has been recently reported that ET receptor antagonists may be used as effective treatment of MED. ET is a potent vasoconstrictor synthesized by the corpus cavernosum smooth muscle cells and endothelium (Andersson and Wagner, 1995). In addition to acting as a vasodilator of corpus cavernosum smooth muscle, NO regulates the expression of endogenously produced ETs (Nehra et al., 1999). The pharmacology of the interaction between nitric oxide and endothelin receptor systems is an area of research still to be resolved.

**Dopamine Receptor Agonists.** Apomorphine is a dopamine receptor agonist known to induce penile erection in men when administered orally (Morales et al., 1995). It has been tested in clinical trials in a sublingual formulation, which overcomes the major side effect of mild nausea. It is expected that it will be effective in a population of patients similar to that of sildenafil (psychogenic and mild-to-moderate vascular MED patients) but without the cardiovascular side effects. A sublingual formulation of apomorphine (Uprima, TAP, Deerfield, IL) has recently been withdrawn from FDA review until the safety profile is further investigated. Although several dopaminergic agents like apomorphine, quinpirole, and 3-PPP can induce penile erections in animals, it is unclear which dopamine receptor subtype mediates the proerectic effect (Vallone et al., 2000).

**Future Prospects**

The development of noninvasive or minimally invasive routes of administration is a key issue in the drug discovery area for the treatment of MED. While a number of reports focus on the use of topical agents to treat erectile dysfunction, success using this route of administration has been limited. Topical PGE$_2$, papaverine, and nitroglycerin have been tested (Nehra et al., 1999). In most of these limited clinical trials, penile blood flow increased and most subjects reported tumescence, but the number of subjects responding with erections sufficient for vaginal penetration was low and in most instances indistinguishable from the placebo. These results are consistent with the problem of drug delivery through the tunica albuginea. Recently, iontophoresis has been tested using an intrarethral catheter. While this report is promising, further studies are necessary to determine whether this means of delivery is effective as a minimally invasive method of treatment.

Oral agents have the advantage of convenience but the disadvantages of systemic side effects. Since the penis is a vascular organ, many of these side effects center around vascular liabilities such as hypotension and incidence of myocardial infarction. There are three PDE5 inhibitors in late stages of clinical development (IC351, ICOS/Lilly; vardenafil, Bayer; E8010, Eisai Pharmaceuticals). Any cGMP-based therapy will have to contend with liabilities of nitrate-based heart disease medications.

Research defining the peripheral pathways of erectile physiology and investigating the pathogenesis of erectile dysfunction has led to the recognition of a predominant vascular basis for organic male sexual dysfunction, while the role of the central nervous system is just beginning to emerge. These scientific advances have laid the foundation for the advent of new treatments. Significant advances in the research of erectile dysfunction indicate that vascular disease appears to exacerbate the changes in corpus cavernosum structure seen with aging. The recent availability of new oral and minimally invasive medications offers the possibility of multiple pharmacological approaches for the treatment of MED. The new frontier of understanding the central control of erection is still in its infancy, and future research into CNS regulation of erection may lead to novel, safer, and efficacious pharmacotherapies.

**References**


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