Endocrine, *vol. 23, nos. 2–3, 167–176, March–April 2004* 0969–711X/04/23:167–176/$25.00

Therapy of Erectile Dysfunction

Potential Future Treatments

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Current research on the development of new medical treatments of erectile dysfunction (ED) fall essentially into two main types of approaches: (1) the traditional strategy based on compounds acting to induce an erection on demand, without modifying the underlying pathologic alterations that lead to ED; and (2) more novel agents not inducing an erectile response but aiming for a long-term correction of either the defect in cavernosal tissue integrity responsible for functional impairment, or the impairment *per se* of the cavernosal tissue function. In the first approach, new phosphodiesterase inhibitors (either more potent and specific than the clinically available ones or harboring nitric oxide–releasing structures), soluble guanylate cyclase activators, Rho kinase inhibitors, as well as centrally active agents stimulating hypothalamic dopamine or melanocortin receptors, combinations of different types of drugs, and new facilitators of tissue uptake of active agents, are being investigated and some may soon be applied clinically. In the second approach, the first type of correction comprises regulators of endogenous cell number and integrity and extracellular matrix turnover (inhibitors of apoptosis and fibrosis, neurotrophic and angiogenic factors), testosterone, and tissue and cell explants (nerve and smooth muscle grafting, adult pluripotent cells), whereas the second includes in vivo gene therapy with different genes and vectors, and ex vivo gene therapy, combining gene transfer with stem cell implants. This second approach requires extensive laboratory research prior to clinical translation but may provide a means to cure ED. The current status and future directions of these strategies are discussed.

Key Words: Corpora cavernosa; tunica albuginea; gene therapy; smooth muscle; testosterone.

Biochemical Pathophysiology of Erectile Dysfunction

Many of the current approaches to the therapy of erectile dysfunction (ED) have arisen from basic research on the biochemical pathways and physiology of both the neurotransmission and cavernosal smooth muscle relaxation that triggers the erectile response *(1–6)*. This erectile process is essentially elicited by the nonadrenergic-noncholinergic release of nitric oxide (NO) in the nerve terminals of the penis on sexual stimulation by activation of penile neuronal nitric oxide synthase (PnNOS), an nNOS variant. This NO stimulates guanylyl cyclase in the smooth muscle of the cavernosal trabeculae to produce cyclic guanosine 5'-monophosphate (cGMP), which in turn activates protein kinase G (PKG), decreasing phosphorylation of myosin regulatory light chain through reduction of cytoplasmic Ca^{2+}, and consequent relaxation (Fig. 1). In turn, acetylcholine-mediated and flow sheer stress–mediated processes are hypothesized to stimulate the endothelial NOS (eNOS) within the endothelial lining of both the trabeculae cisternae and the vasculature, and this may contribute to smooth muscle relaxation through additional NO synthesis. cGMP is degraded by the cGMP-dependent phosphodiesterases (PDEs), specifically the PDE5A isoform within the penis, with cross talk between cyclic adenosine monophosphate (cGMP) and cAMP at different levels of their metabolism. Although other relaxants, such as vasointestinal peptide, calcitonin gene–related peptide (CGRP), adreno-medulline, and adenosine, are present in the penis, they are not considered to play a significant role in the normal erectile response.

To trigger cavernosal smooth muscle relaxation, NO has to counteract the contractile tone of this tissue *(2,4)* that is maintained mainly by noradrenaline that acts on α1-adrenergic receptors, activating PKC through phospholipase C and inositol phosphate 3/diacylglycerol pathways, promoting Ca^{2+} increase, myosin phosphorylation, and contraction (Fig. 2). Endothelin 1 (ET-1), and to a lesser extent ET-2 and -3, binding to ET_A and ET_B receptors, is assumed to maintain the contractile tone through a similar mechanism, but the role of these peptides is not well established. A new class of downstream contractile effectors has recently been characterized—the Ca^{2+} sensitizers, mediated by guanosine 5'-triphosphate (GTP)–binding proteins—that triggers

Received September 15, 2003; Revised November 14, 2003; Accepted November 14, 2003.

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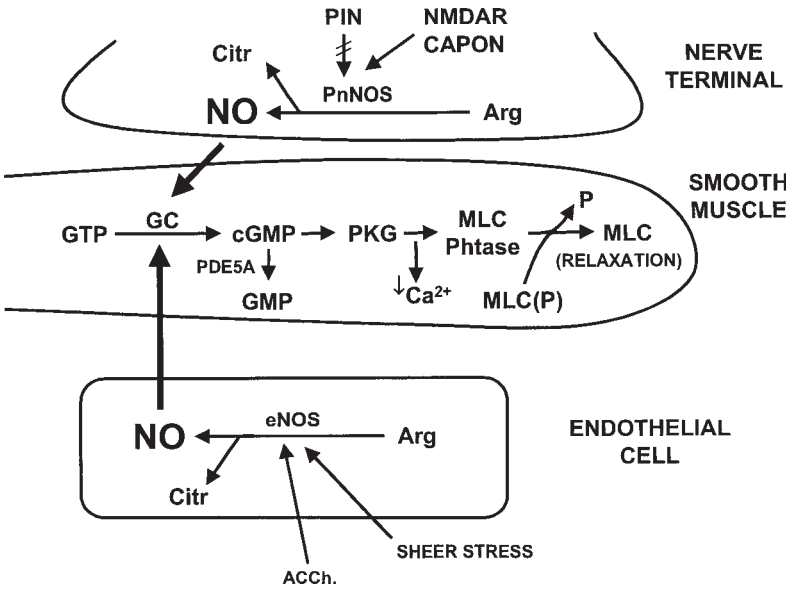

Fig. 1. Schematic representation of main biochemical pathways operating in relaxation of penile corpora cavernosa smooth muscle. NO, nitric oxide; Citr, citrulline; Arg, L-arginine; PnNOS, penile neuronal NOS; PIN, protein inhibitor of NOS; NMDAR, NMDA receptor; GC, guanylyl cyclase; PDE5A, phosphodiesterase 5A; PKG, protein kinase G; MLC Phtase, myosin light chain phosphatase; MLC(P), phosphorylated myosin light chain; eNOS, endothelial NOS; ACCh, acetylcholine; Ca^{2+}, cytoplasmic Ca^{2}; cGMP, cyclic guanosine 5'-monophosphate; GTP, guanosine 5'-triphosphate. For the sake of simplification, some intermediate or ancillary steps are omitted (see refs. *2–4*).

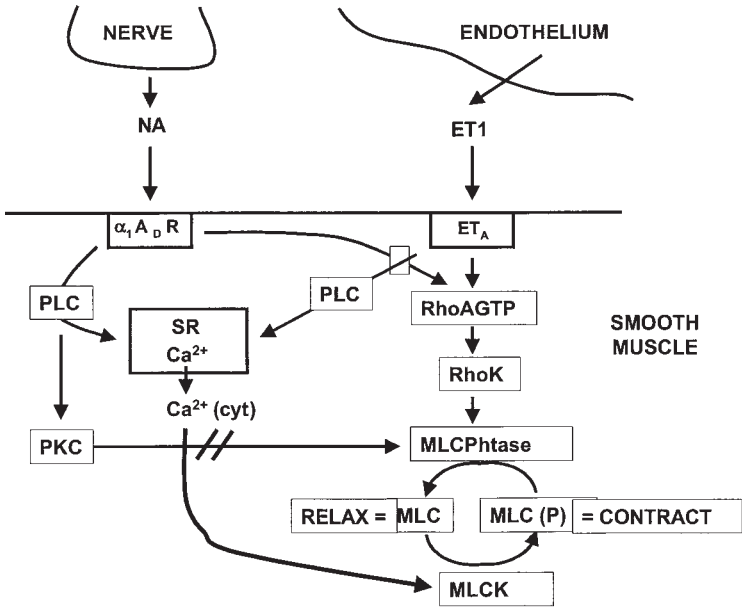

Fig. 2. Schematic representation of main biochemical pathways operating in contraction of penile corpora cavernosa smooth muscle. NA, noradrenaline; ET1, endothelin 1; $\alpha 1A_DR$, α1-adrenergic receptor; ET_A, endothelin receptor A; PLC, phospholipase C; PKC, protein kinase C; SR, sarcoplasmic reticulum; Ca^{2+}, cyt, cytoplasmic Ca^{2+}; RhoAGTP, GTP-bound RhoA; RhoK, RhoA kinase; MLCPhtase, myosin light chain phosphatase; MLC(P), phosphorylated myosin light chain; MLCK, myosin light chain kinase. For the sake of simplification, some intermediate or ancillary steps are omitted (see refs. *2* and *4*).

a higher force/intracellular Ca^{2+} ratio than the one induced by K^+ depolarization. α1-Adrenergic and ET receptor agonists stimulate Ca^{2+} sensitization, whereas NO inhibits this process. The mediator of Ca^{2+} sensitization is Rho kinase activation by GTP-RhoA, which inhibits myosin light chain phosphatase, raising the levels of phosphorylated myosin, and subsequent contraction.

In addition to these pathways operating in the effector organ (i.e., the penis), the central control of erection at both the brain and spinal cord is also based on nitrergic transmission *(5,6)*. The activation of dopaminergic and oxytocinergic receptors in the hypothalamic areas of the male rat responsible for the control of erectile, ejaculatory, and reproductive functions (paraventricular nucleus [PVN],

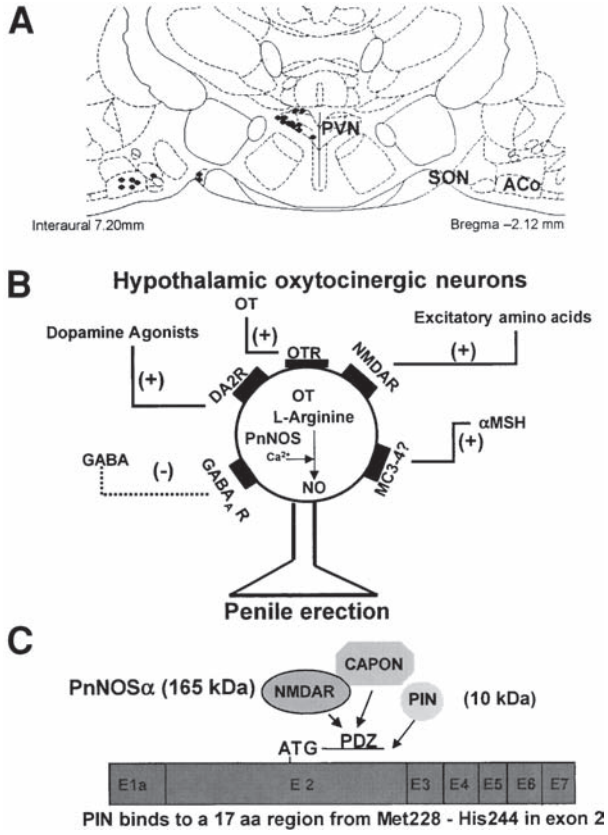

Fig. 3. Schematic representation of main biochemical pathways operating in hypothalamic control of erection through nitrergic neurotransmission. (**A**) Outline of hypothalamus at paraventricular nucleus (PVN) and supraoptic nucleus (SON) levels, indicating the presence in the PVN of PnNOS-containing neurons, mainly at magnocellular, and also at parvocellular, regions. Some PnNOS colocalizes with oxytocinergic neurons. PnNOS neurons are also present in the anterior cortical amygdaloid nucleus (ACo), and in the medial preoptic area (MPOA; not at this plate). (**B**) Oxytocinergic (OT) neurons in PVN showing oxytocin receptors (OTR); dopamine 2 receptors (DA2R); NMDA receptors (NMDAR); GABA receptors ($GABA_AR$); melanocortin receptors 3 or 4 (MC3–4); γ-aminobutyric acid (GABA); α-melanocyte stimulating hormone (αMSH); +, stimulation; –, inhibition. (**C**) Representation of interaction of regulatory peptides with PnNOS: E, exon; PDZ, binding site for regulators; kDar, kilodaltons (size) (see refs. *5–9*).

and medial preoptic area [MPOA]) stimulates NOS and produces NO that is essential for triggering erection through chemical or electrical stimulations of those areas *(5–8)*. The same occurs in the sacral spinal cord *(7)*. In addition, proteins that bind to nNOS and regulate its activity, such as the *N*-methyl-D-aspartate (NMDA) receptor and the protein inhibitor of NOS (PIN), have been located colocalizing with nNOS in the same hypothalamic neurons *(7,8)*, resembling the situation in the nitrergic nerve terminals of the penis *(9)* (Fig. 3).

At the molecular and cellular levels, ED may result essentially from: (1) insufficient production of NO as endogenous relaxant of the corporal smooth muscle, by either the neurally located PnNOS or the eNOS, possibly compounded by an increase in contractile tone through mainly α1-adrenergic receptor–mediated pathways; and (2) the loss or damage of the penile nerves responsible for nitrergic neurotransmission, or of the target corporal smooth muscle combined with an increase in collagen fibers (fibrosis) that leads to an impaired compliance of the smooth muscle and defective vasorelaxation. The most prevalent type of ED, vasculogenic ED, is probably owing to a combination of the latter process with loss of smooth muscle cells (SMCs) and fibrotic degeneration within the corpora, induced mainly by aging, diabetes, and/or vascular disease *(10–12)*. Neuropathic ED can also involve the first process, but in combination with loss or damage of the penile nerves, such as in diabetes and after radical prostatectomy *(13–15)*. No defect in the central control of erection has so far been properly characterized at the cellular or biochemical level, or even associated with a specific type of neuropathy. However, aging-related expression of inducible NOS (iNOS) appears to play a neurotoxic role through the production of peroxynitrite and induction of apoptosis in hypothalamic neurons in the PVN and MPOA, specifically oxytocinergic and gonadotropin-releasing hormone containing neurons *(16,17)*.

This review focuses on novel approaches and concepts derived from this knowledge that are promising for the pharmacologic management of ED, rather than on results of clinical trials for drugs in current use. Most of these innovative agents or modalities of pharmacologic treatment are still at the level of animal experimentation or without proven clinical record, but some are soon likely to be translated to the clinic. Some of these treatments continue the current pharmacologic approach based on "on-demand" administration to relax the cavernosal smooth muscle and induce an erection and, therefore, can be considered palliative and noncurative of the problem. The most innovative and promising of these approaches are, however, those that aim to correct the biochemical and tissue dysfunction that underlies most types of ED, and conceptually they may be considered curative in scope. The latter category includes agents that aim to restore nerve and smooth muscle architecture that would lead to the normal functioning of the erectile mechanism, or target the biochemical alterations responsible for an impaired functional response.

Promising Novel Pharmacologic Approaches to Stimulate Cavernosal Relaxation and Erectile Response in ED

New PDE Inhibitors and Centrally Acting Drugs

The advent of sildenafil and its considerable success *(18, 19)* marked a stage in the treatment of ED that appeared to slow down and give pause to the development of additional novel therapies for this condition. However, it soon became apparent that a sizable fraction of patients (35–50%) did not respond to this drug *(20,21)*, and, therefore, the search for new drugs became energized, mainly for analogs of

PDE5 inhibitors and hypothalamic-acting drugs that affect the central control of erection. Drugs in the first group, including tadalafil *(22)* and vardenafil *(23)*, act through the same mechanism as sildenafil by elevating cGMP concentrations preferentially in the penis; therefore, an approach based on PDE5 inhibition may not substantially improve the therapeutic efficacy over sildenafil, or the range of ED conditions responsive to this treatment. The chemical conjugation in the same compound of both a PDE5 inhibitor and an NO donor may, however, improve efficacy, since it would provide a sustained production of NO combined with the inhibition of cGMP breakdown, and preliminary results are encouraging *(24)*.

The second group of oral, intranasal, or sublingual drugs either with FDA approval or in clinical trials, is based essentially on apomorphine and related agonists of dopamine 1–2 receptor agonists, such as oxytocin *(5,6,25–27)*, acting via the direct central nitrergic mechanism discussed in the previous section that elevates NO release in the hypothalamus, or through melanocortin receptors *(28,29)*. However, apomorphine does not seem to act at the spinal level through oxytocin receptors *(30)*, and this and related drugs may not always be operating through identical receptors in the hypothalamus and spinal cord. The main conceptual problem for these drugs is that, in principle, a stimulation of the central control of erection may not efficiently counteract a defective relaxation of the corpora cavernosa owing to venoocclussive dysfunction derived from smooth muscle fibrosis, the most prevalent form of ED, or nerve damage after radical prostatectomy. In addition, clinical efficacy of existing centrally acting drugs does not seem to be as high as for PDE inhibitors *(31)*. Therefore, it is logical to assume that novel approaches for centrally acting drugs may be focused on two aspects: (1) utilization of higher doses by reduction of side effects (vomiting) by independent targeting of erectile centers without affecting emetic centers, through better receptor specificity; and (2) identification of novel receptors or peptides that would be more efficient than the ones already described in regulating nitrergic neurotransmission and nNOS activity in the brain.

Other Agents

Since studies in rats have shown an increase in aging-related apoptosis in oxytocinergic neurons of hypothalamic regions involved in the control of penile erection, associated with an increased production of the iNOS *(16,17)*, it may be speculated that a certain degree of damage of the hypothalamic control of reproductive behavior, and specifically of erection and ejaculation, occurs with aging. Neuroprotective drugs that protect hypothalamic neurons involved in erectile function from this process, such as selective estrogen receptor modulators *(32,33)*, may also be developed, but it can be predicted that centrally acting drugs may be more efficacious for the improvement of ejaculatory disorders than of erection.

Other categories of agents that are being tested experimentally comprise the new soluble guanylyl cyclase activators that stimulate the "receptor" for NO, elevating cGMP synthesis *(34–37)*, and the Rho kinase inhibitors that block Ca^{2+} sensitization *(2,4,38–42)*, in both cases causing smooth muscle relaxation. It is not clear whether the guanylyl cyclase activators are sufficiently specific on cGMP synthesis in the penis as to avoid affecting the general vasculature. Although a rho kinase inhibitor, Y-27632, has been able to increase the neurogenic erectile response to cavernosal nerve stimulation in spontaneously hypertensive, castrated, and diabetic male rats, no significant effect was observed in the widely used aging rat model of ED, and more work needs to be done to conclude whether as single agents these inhibitors are better than existing therapies.

Oral long-term supplementation with L-arginine, the substrate of NOS, was shown to correct ED in aged rats *(43)*, and the same encouraging results have been observed in vascular dysfunction in both men and laboratory animals *(44)*. Human studies are needed to conclude whether this treatment may be warranted for ED, although it may not be an "on-demand" administration approach producing immediate erection, as all the ones above. Hydroxyarginine, an intermediary of NO metabolism, is currently being investigated as a smooth muscle relaxant in the corpora cavernosa *(45)*. Chemical NO donors as single vasodilating agents may pose risks of priapism and hypotension.

Drug Combinations and New Routes

The combination of drugs currently in clinical use offers some interesting possibilities, in a way reviving the approach that was prevalent 10 yr ago with intracavernosal "trimixes" containing prostaglandin E_2 (PGE_1) as a stimulator of cAMP synthesis by binding to e-prostanoid receptors, papaverine as a nonspecific inhibitor of PDE increasing both cAMP and cGMP levels, and phentolamine as an α1-adrenergic receptor blocker *(46)*. Although no clear advantages over single-drug treatments were obtained in small clinical trials, and there were even some risks of undesirable side effects (priapism, hypotension), the concept is appealing, since it is widely applied in other areas such as cancer and cardiovascular disease therapy. It is based on the combination of drugs acting by different mechanisms with durable efficacy, and it is hoped without overlapping adverse effects *(1–6)*.

Such combinations may be oral or sublingual but may also include oral/intracavernosal, and they involve cAMP- and cGMP-dependent PDE inhibitors (sildenafil) with α1-adrenergic receptor blockers (doxazosin), cAMP-dependent PDE inhibitors that may act through both PKG and PKA activation, centrally acting dopamine receptor 1–2 agonists (apomorphine) with PDE5 inhibitors or α1-adrenergic receptor blockers *(47)*, activators of guanylyl cyclase (YC1) and PDE5 inhibitors or NO donors *(34)*, PDE5 inhibitors (sildenafil) and (phentolamine), PDE inhibitors (sildenafil) and NO generators (L-arginine), Rho kinase inhibitors and

relaxant agents, PDE4 inhibitors and PGE_1 as cAMP-dependent vasodilator *(48)*, and others. However, because of costs and potential complications, it is not known whether any combination may prove to be more effective than the current single drugs.

Finally, an approach that is being actively pursued is a noninvasive direct administration of the active agent into the penile corpora cavernosa that could ensure retention of the active compound in the corpora with little systemic dissemination, comparable to the intracorporeal injection. In addition, new facilitators of uptake of the agent into the corpora smooth muscle by traversing the tunica albuginea that would allow external application somewhere onto the shaft of the penis are being developed *(49–51)*.

Promising Approaches to Correct the Underlying Defect in Cavernosal Tissue Integrity in ED

Regulators of Endogenous Cell Number and Integrity and Extracellular Matrix Turnover

The loss of smooth muscle and the progressive fibrosis occurring with aging, and possibly during diabetes and vascular disease, has been demonstrated in both men and rats, and in men it is the most likely cause for venous leakage owing to the inability of the corporal tissue to compress the cavernosal veins shut against the tunica albuginea *(52,53)*. Since it is clear that in cardiovascular tissues both collagen fibers and cells are in active turnover *(54–56)*, it is plausible that by modifying this rechange or remodeling toward the reduction of collagen deposition and the maintenance of SMC number venous leakage may be corrected or even prevented from occurring. Similarly, in diabetic neuropathy and after radical prostatectomy, the penile nerves involved in erection may be damaged and neuropathic ED may ensue *(14,15)*; therefore, the aim of such therapy would be to replace the nonfunctional neural tissue. As such, this modality of therapy trying to preserve or restore the normal cavernosal tissue architecture, and by extension its function, would be "curative" rather than "palliative." In this sense, this approach would have aims similar to the ones of gene therapy (discussed later).

The prevention or reversal of excessive collagen deposition in the corpora cavernosa can be addressed with drugs that inhibit collagen synthesis, or stimulate its breakdown, through the manipulation of the complex interplay of matrix metalloproteinases and their inhibitors *(54,57,58)*. Many of the concepts derived from the treatment of tissue fibrosis and vascular dysfunction can be extrapolated into this area, and perhaps the most enticing is the counteraction of oxidative stress created by the production of reactive oxygen species (ROS) as a main factor of aging degeneration and fibrosis in vascular tissue *(59,60)*.

In the penis, novel strategies to reduce the Peyronie-like fibrotic plaque in the tunica albuginea of the rat *(61–67)* or fibrosis of the penile artery media *(68)* through counteraction of oxidative stress can in fact be extrapolated to the corporal smooth muscle tissue, and NO generators (L-arginine, NO donors, gene therapy with iNOS), PDE inhibitors (pentoxifylline, sildenafil), and antioxidants of different types may be applicable in long-term treatments, not simply to induce smooth muscle relaxation but to reduce fibrosis. These compounds appear to act by reducing ROS levels and inhibiting collagen synthesis *(61–66)*, but they may also stimulate collagen degradation. Agents that in turn break collagen crosslinkage by advanced-end glycation products, which are metabolites that are increased in the penile tissues in aging and diabetes, may also be useful in helping to reduce cavernosal fibrosis. Some of them, such as 3-phenyacyl-4,5-dimethylthiazolium chloride (ALT-711), are currently in clinical trials for vascular disease *(69,70)*.

An area that is still in its infancy but is very promising is the replacement of damaged or lost tissue, in particular, smooth muscle and/or nerves. For smooth muscle, this can theoretically be approached by manipulating existing corporal SMCs to increase their rate of replication, a process that occurs in the vascular media during restenosis, or by reducing apoptosis, through biologic or chemical modulators, e.g., stimulating tissue remodeling *(71)*. This may occur by directly reducing extracellular matrix deposition rather than by affecting the SMCs themselves, because of the mutual interaction between both compartments *(55)*. Vascular endothelial growth factor (VEGF) has been recently shown to restore erectile function by intracavernosal injection in rat models of castration-induced venoocclusive dysfunction *(72)*, hyperlipidemia *(73)*, and traumatic arteriogenic ED *(74)*. This appears to occur through NO- and cytokine-mediated mechanisms probably triggering smooth muscle replication in the cisternae themselves, since no clear demonstration of angiogenesis has been provided.

Axonal nerve regeneration in the corpora cavernosa after surgical damage, injury, or progressive atrophy, can be induced from proximally located neuronal bodies, by neurotrophic factors *(75)*. These factors, mainly brain-derived neurotrophic factor (BDNF) *(76–78)*, acidic and basic fibroblast growth factor *(79,80)*, and even VEGF *(72–74,81)*, restore damaged nerves in rodent models of neurogenic ED. Other neuroprotective agents not belonging to the growth factor class may be promising, such as the immunoligand FK506 that protects penile innervation from degeneration following nerve-crush injury and preserves erectile function *(82,83)*. FK506 binds to a specific immunophilin protein (FKBP12), but the mechanisms of the neurotrophic effects of immunophilin ligands are unknown.

Androgen Treatment of Aging-Related ED

An area of endocrine treatment that is being revisited with new approaches based in part on the aim of restoring corpora cavernosal tissue integrity and function rather than inducing a vasorelaxing effect on demand is the administration of testosterone supplementation in two modalities:

(1) for clearly hypogonadal men *(84–88)*, and (2) for men failing treatment with sildenafil who have low but not necessarily hypogonadal levels of testosterone *(89–91)*. Animal experiments for this treatment are based on the fact that in rats castration induces a severe reduction in the erectile response to electrical field stimulation of the cavernosal nerve, and this is accompanied by a decrease in NOS activity *(89,92–97)* and an increase in apoptosis in the cavernosal smooth muscle and collagen deposition, causing smooth muscle fibrosis *(98,99)*. These effects are reversed by testosterone and some of them by dihydrotestosterone (DHT) *(92)*. Rho kinase and other contractile factors leading to Ca^{2+} sensitization are upregulated by castration *(2,38)*.

Castration-associated ED is clearly vasculogenic, since venous leakage is demonstrated by cavernosometry and related techniques *(100,101)*. Venous leakage induced in the rat by castration *(99)* and other procedures *(102–104)* is associated with fibrosis. In addition, there is a marked reduction in the mass of the sexually dimorphic perineal striated muscles, the bulbospongiosum and ischiocavernosum muscles, which in the rat are responsible for both the final phase of erection and rigidity *(105,106)*. Androgens counteract most of these changes and, in addition, stimulate cAMP levels, which may favor cavernosal smooth muscle relaxation.

Experiments in rats showed that aging and diabetes mellitus are associated both with a decreased penile erectile response and a reduction in serum testosterone levels *(107–112)*, and with fibrosis of the trabecular smooth muscle and the media of the penile arteries *(9,68,107,113–115)*, so in theory testosterone supplementation in elderly or diabetic men with subnormal serum levels may be warranted. However, tissue androgen dependence and responses may be species related, since perineal muscles in men may not be as significant for rigidity as in rats, and, by contrast, skeletal muscle mass appears to be more sensitive to androgens in men than in rats, so results cannot be directly extrapolated. Double-blind placebo-controlled studies of long-term testosterone supplementation by gels and patches in a larger number of patients with well-defined suboptimal testosterone levels are needed, as well as a more precise characterization of ED with cavernosometry, and Rigiscan to measure penile rigidity.

Tissue and Cell Explants

The most feasible strategy to restore damaged cavernosal tissue integrity is implantation of autologous tissue explants or disaggregated cultured cells. The first approach is being explored mostly for reconstructive surgery, utilizing scaffolds of collagen lattices in which autologous cavernosal smooth muscle and endothelial cells are microencapsulated and grown into organized tissue-like structures. Although it has been shown to generate a partially functional tissue in a rabbit model *(116)*, this procedure may not be warranted for most ED cases, in which the integrity of the smooth muscle and endothelium may be only partially damaged. In addition, the reconstruction of the correct gap junctions and trabecular structure in vitro, prior to implantation, is a major hurdle in this approach. The nerve-grafting approach using the genitofemoral nerve, initially introduced by Walsh's group in 1990 in rats and humans, without significant effects in men on postradical prostatectomy ED, has recently been revived with encouraging results (see ref. *75*). Similarly, direct transplantation of pelvic ganglion to the corpora cavernosa is promising in rats *(117)*. However, since these are essentially surgical procedures that do not involve cell culture or pharmacologic modulation of the tissue, it is not within the scope of this review.

A more feasible procedure for the corpora cavernosa smooth muscle is based on monolayer cultures of cells derived from normal tissue that after expansion are then trypsinized and injected as cell suspensions into the impaired corpora cavernosa of the donor patient. The expectation is that they will infiltrate among the endogenous tissue and naturally restore their normal cell-to-cell interaction. However, autologous regular SMCs may not replicate well or may be rejected, as occurs with many cell implants, particularly in degenerating aged host tissue *(118)*. To overcome these problems, utilization of adult pluripotent cells (PPCs), or "stem cells," able to evolve into different cell lineages on selective stimulation, is being actively investigated for implants *(118,119)*. These features may allow for higher survival and replication rate on implantation, and a specific tune-up of their differentiation to the paracrine cues of the host target tissue. These primary cultures avoid the problem with existing cell lines with stem cell features that may replicate indefinitely and cause dysplasias or tumors. The existence of PPCs in the adult bone marrow has long been recognized, but stem cells have only recently been detected and isolated from a variety of other tissues and organs.

Perhaps the most clinically relevant tissue sources, because of easily available biopsies, are the skin, where mouse dermal fibroblasts implanted into injured skeletal muscle have been shown to fuse their nuclei into the muscle fibers *(120)*, and the skeletal muscle itself, which contains PPCs that may evolve into hematopoietic cells, bone cells, cardiomyocytes, or even myofibroblasts *(119,121,122)*. Skeletal muscle PPCs are the subject of intense interest as a novel vehicle for gene therapy, particularly in the cardiovascular system. Specifically in the case of ED, a fraction of mouse primary myoblasts enriched in stem cells (muscle-derived stem cells [MDSCs]) has been tested for the ability to regenerate peripheral nerves for ED after postradical prostatectomy, in a rat model of cavernous nerve transection *(123)*. LacZ-prelabeled MDSCs persisted 2 wk after injection in the implant region, and ED was partially corrected. However, this was only a preliminary approach hampered by the use of a heterologous system, and the lack of characterization of cell differentiation and a long-term follow-up. Bone marrow–derived stem cells are being investigated for the reconstitution of penile tunica albuginea *(124)*.

Therefore, considerable work needs to be done using the appropriate differentiation markers to conclude that MDSCs can really differentiate into nerve cells in the corpora cavernosa. Similarly, MDSCs from muscle biopsies from ED patients, and other stem cells, such as fat-derived stem cells obtained by liposuction *(125)*, can theoretically be converted into SMCs after reimplantation in the same patient, for replacement of impaired smooth muscle in aging-derived ED. This is an area of great promise, where androgens and other hormones may play a role as adjuvants in cell transplantation by modulating the implanted stem cell differentiation into cavernosal smooth muscle for vasculogenic ED or, for neuropathic ED, into nerve cells. Dexamethasone, insulin, and insulin-like growth factors-1 and -2 are part of some of the in vitro culture media utilized for selective lineage differentiation of stem cells, and it has been recently shown that testosterone and DHT can stimulate myogenesis and inhibit adipogenesis in a mouse mesenchymal cell line *(126,127)*.

Promising Approaches to Correct the Underlying Defect in Cavernosal Tissue Function in ED

In Vivo Gene Therapy

Proof of the concept that ED can be ameliorated via the pharmacologic transfer to the corpora cavernosa of a gene related to the control of erection *(128–130)* was based on the principle that NO is the chemical mediator of penile erection, and, therefore, NOS gene transfer was tested in the rat model of aging-related ED. The rationale was that a higher NO output may overcome the defective cavernosal smooth muscle compliance and/or a putative excessive contractile tone present in the aged animal. Of the three main isoforms of NOS, iNOS was selected because although iNOS is not normally expressed in the penis, it is easily induced in rat penile SMCs in culture utilizing a cytokine mix *(131)*, and because human and rat iNOS cDNA had been cloned from the penis *(132)*. By injecting a plasmid containing iNOS cDNA in a liposomal preparation, it was shown that expression of the recombinant iNOS protein occurred in the corpora cavernosa and corrected the defective erectile response of the aged rat for at least 10 d, without side effects.

eNOS *(133–135)* was then shown to be also pharmacologically active for correcting ED in the aged and the diabetic rat, by using a replication-defective adenoviral vector aimed at prolonging the persistence of the recombinant construct, although measurements of erectile response were only conducted at 1–5 d. The cDNA for the third NOS isoform, nNOS, and specifically the PnNOS variant cloned from both the rat and human penis *(136–138)*, corrected for at least 17 d ED in aged rats. This variant is the only nNOS present in the penis, and one of its splicing subvariants (the β form) is the one that is expressed in the nNOS knockout mouse and is assumed to be responsible for the persistence of erectile function in this animal *(137)*. The combination of a helper-dependent adenoviral virus and a new procedure (in vivo electroporation) to increase uptake of the cDNA construct allowed a comparatively low viral load to be efficacious. In addition, antisense cDNA directed to block the expression of PIN, an inhibitor of nNOS *(7,9)*, has been shown to correct ED in the aged animal for at least 30 d *(139)*. These contributions supported the hypothesis that NOS gene therapy, irrespective of the isoform, is effective in improving erectile function, and that a virus rather than a plasmid may be an adequate vector.

The efficacy of gene therapy to ameliorate ED has also been extended to other genes related to cavernosal relaxation, such as maxi K^+ channels (hSlo) *(140–142)*; CGRP *(143)*; trophic factors, such as VEGF *(144,145)*, BDNF *(73,146)*, and neurotrophic factor *(147)*; and to antioxidant enzymes such as superoxide dismutase *(148)*, or to block contractile factors such as Rho kinase with a dominant negative mutant that reduced RhoA/Rho-kinase signaling *(42)*. Some of these studies described very short effects (less than 1 wk), but VEGF and BDNF were administered as AAV constructs, with reported effects as long as 2 mo. The plasmid construct of the maxi K^+ channel cDNA was given to 9-mo-old rats as "naked DNA" at high doses (100–1000 μg) and stimulated the erectile response accompanied by persistence of the injected plasmid for at least 6 mo *(142)*. However, in that study the control rats did not display the progressive type of ED that occurs with aging, so the efficacy for long-term correction of this condition with plasmid constructs needs to be established more conclusively. Although these vectors do not pose some of the potential regulatory problems of viral vectors, evidence from the literature for plasmids carrying other genes that were applied to vascular conditions is not convincing in terms of efficiency and length of response.

Ex Vivo Gene Therapy

A promising approach to circumvent some of the technical problems affecting in vivo gene therapy is the ex vivo transfection of cell cultures with the desired gene construct, which are then transplanted into the corpora cavernosa. The expectation is that these cells would either disperse throughout the tissue or otherwise concentrate in the region of injection and act as a "factory" for the intracavernosal secretion of the recombinant protein or the diffusion of its product. This strategy would target the vector specifically to the desired cell type and organ and may minimize the viral construct load needed. It would also avoid circulating viruses and allow the use of strong viral promoters instead of weaker tissue-specific promoters, increasing the production of the recombinant protein. Vectors that only transduce replicating cells, such as retrovirus, may then be applied *(149–151)*.

Recently, an explant of mouse skeletal muscle fibroblasts transfected with the iNOS cDNA into the corpora cavernosa of the rat stimulated for 2–7 d the erectile response *(152,153)*,

but because of conceptual and technical concerns, it is difficult to assess the success of this approach. More conceptually acceptable is the use of stem cells, as in the case of the MDSCs discussed earlier, but in the only single study so far reported, a nonpharmacologically significant gene expressing the reporter β-galactosidase was transduced *(123)*.

Future Directions of Gene Therapy

AAV vectors can ensure gene expression for years *(154)*, but there are still technical problems in the preparation of these constructs that need to be resolved, although these constructs have been applied successfully for gene therapy of ED with some of the genes already mentioned *(146,147)*. The more practical currently available helper-dependent, replication defective, "gutless" AdV vectors *(138,143,148)* may still allow expression for only months, and novel derivatives with more prolonged expression are being developed *(155)*. Lentivirus and herpesvirus are also potential vectors *(147,156)*, and preliminary experiments have been reported with the latter viral vectors in the rat corpora cavernosa *(147)*.

Utilization of tissue-specific gene promoters to allow for preferential expression of the recombinant DNA in a given tissue, irrespective of disseminated uptake, is also being explored. The neuronal specific enolase *(157)* and α smooth muscle actin *(158)* promoters are activated, respectively, in neurons and SMCs and may be combined with the restricted delivery approach to improve selectivity of expression in these cavernosal tissues. The study of several endogenous factors that control PnNOS activity, such as PIN or carboxy-terminal PDZ ligand of mNOS (CAPON) *(7,9)*, may spur the design of gene transfer approaches based on downregulating the expression of these proteins with ribozyme, siRNA, or antisense approaches *(159,160)*, such as for PIN *(139)*, or on competing with their binding to PnNOS based on the use of cDNAs encoding proteins that may interfere with this interaction. Finally, the availability of novel promoter casettes where the recombinant cDNA is placed under a promoter that is regulated by very low nonhazardous doses of a drug, such as doxycycline, ecdysone, or RU486 *(161,162)*, may be applicable. In this way, production of the recombinant protein in the corpora cavernosa may be activated only when the drug is given. Suspension of the drug treatment stops further expression, and the cycle may be repeated.

These improvements, combined with electroporation, tissue uptake facilitators, and other strategies, may help meet the objective of minimizing viral load and side effects, while still prolonging efficient expression and pharmacologic activity. As to ex vivo gene therapy, considerable work needs to be conducted, in terms of stem cell technology, selection of viral construct and gene, and implant survival, to decide whether this approach is feasible and more efficacious than the in vivo procedures. All these modalities of gene therapy are exciting possibilities, with considerable experimental evidence in animal studies, and may soon begin to be translated to the clinic.

Acknowledgments

This work was funded by grants NIH R01DK53069 and G12RR-03026, and The Edythe and Eli Broad Foundation.

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