Modulation of Soluble Guanylate Cyclase for the Treatment of Erectile Dysfunction

Nitric oxide (NO) is the principal mediator of penile erection, and PDE-5 inhibitors are the first-line agents used to treat erectile dysfunction (ED). When NO formation or bioavailability is decreased by oxidative stress and PDE-5 inhibitors are no longer effective, a new class of agents called soluble guanylate cyclase (sGC) stimulators like BAY 41-8543 will induce erection. sGC stimulators bind to the normally reduced, NO-sensitive form of sGC to increase cGMP formation and promote erection. The sGC stimulators produce normal erectile responses when NO formation is inhibited and the nerves innervating the corpora cavernosa are damaged. However, with severe oxidative stress, the heme iron on sGC can be oxidized, rendering the enzyme unresponsive to NO or sGC stimulators. In this pathophysiological situation, another newly developed class of agents called sGC activators can increase the catalytic activity of the oxidized enzyme, increase cGMP formation, and promote erection. The use of newer agents that stimulate or activate sGC to promote erection and treat ED is discussed in this brief review article.

Physiology of Erection and Pathophysiology of Erectile Dysfunction

Penile erection is a complex neurovascular response that involves integration of central nervous system activity and the release of vasodilator factors from nerves innervating the penis and the endothelium of the penile arteries as illustrated in FIGURE 1 (5, 35). Nitric oxide (NO) is the principle mediator of penile erection and is released from nerves innervating the corpora cavernosa and small penile arteries when the stimulus for erection occurs (20, 42, 43, 68). The discovery that NO had an important role in mediating penile erection was made by Ignarro, Rajfer, and coworkers (42, 43, 68). It was observed that relaxation responses in isolated strips of corpora cavernosa from human and rat in response to electrical-field stimulation were attenuated by an inhibitor of NO synthase (NOS) (43, 68). In addition, it was observed that corpora cavernosal relaxation responses to electrical-field stimulation were enhanced by zaprinast, a PDE-5 inhibitor (68). This important discovery and the observation that the PDE-5 inhibitor had minimal effect on systemic arterial pressure but induced penile erection in patients led to the development of the first PDE-5 inhibitor (Viagra) on the market for the treatment of erectile dysfunction (ED) (6, 16, 17, 34, 45, 62). The observation that a NOS inhibitor markedly attenuated the increase in intracavernosal pressure in response to cavernosal nerve stimulation in the rat demonstrated the important role of NO in mediating penile erection (20). When released from the terminals of nonadrenergic, noncholinergic (NANC) nerves and the endothelium of the corpora cavernosa and small penile arteries, NO binds to the reduced heme iron (Fe^{2+}) on the β-subunit of soluble guanylate cyclase (sGC), which increases the catalytic activity of the enzyme (FIGURE 2) (8). The cGMP that is formed from GTP decreases the intracellular calcium ion concentration in penile smooth muscle by several mechanisms and induces relaxation of the corporal smooth muscle and vasodilation of small penile arteries (5, 53). This results in the sinusoids of the corpora cavernosa filling with blood and increases intracavernosal pressure, which restricts venous outflow. Erection is maintained by compression of small veins of the tunica albuginea. The release of NO from the endothelium of the corpora cavernosa and small arteries is mediated by the activation of M3 muscarinic receptors and an increase in intracellular calcium in the endothelium, which activates endothelial NOS (eNOS), increasing the conversion of L-arginine to L-citrulline and NO (4, 83). The increase in blood flow to the penis increases shear stress, which increases NO release by a flow-mediated mechanism, and a myogenic response in the veins draining the corpora cavernosa all contribute to the development
of penile erection (5). The role of neuronal NOS (nNOS) and NO release from the nerves innervating the penis has been studied in knockout mice and by the use of nNOS inhibitors (41, 76). The release of NO from nNOS-containing nerves can account for most of the NO mediating the erectile response (21, 41, 76). There are at least three components to the cavernosal nerves innervating the penis as shown in FIGURE 1: 1) a NANC component responsible for the release of NO, 2) a component that releases the adrenergic neurotransmitter norepinephrine that has a role in mediating penile detumescence, and 3) a cholinergic component responsible for the release of acetylcholine (ACh) (FIGURE 1) (53). Moreover, recent studies have shown that each of the separate neurons of the cavernosal nerve may contain more than one neurotransmitter. ACh may be colocalized with nNOS, NO, vasopressin, and neuropeptide Y in cholinergic nerve terminals, whereas NANC nerves may contain nNOS and enzymes such as the heme oxygenases (4, 37, 38). The ACh released from cholinergic nerve terminals can act on muscarinic receptors on the endothelium to increase intracellular calcium concentration, which combines with calmodulin to activate eNOS. The NO that is released from the endothelium diffuses into...

![Diagram showing major pathways mediating smooth muscle relaxation and penile erection](http://physiologyonline.physiology.org/)

**FIGURE 1.** Diagram showing major pathways mediating smooth muscle relaxation and penile erection. NO is produced in endothelial cells by eNOS, which converts L-arginine in the presence of oxygen to NO and L-citrulline in response to increases in intracellular calcium in response to increased shear stress or the binding of ACh from cholinergic nerves to muscarinic receptors on the corporal endothelium, which can be blocked by atropine. NO is also produced by the nonadrenergic, noncholinergic (NANC) nerves in response to central or peripheral nerve stimulation and subsequent influx of calcium through N-type calcium channels. The increase in intracellular calcium results in the formation of calcium-calmodulin complexes, which activate nNOS. NO released from NANC nerve terminals or endothelial cells diffuses into corporal smooth muscle and bind to its intracellular receptor, which is sGC. Binding of NO to sGC results in an increase in the conversion of GTP to cGMP. The increase in intracellular cGMP concentration results in activation of protein kinase G (PKG). Activation of downstream targets of PKG results in an overall decrease in intracellular calcium by increasing uptake of intracellular calcium into stores (sarcoplasmic reticulum) and inactivating membrane calcium influx channels. The activity of PKG can be prolonged by inhibiting the breakdown of cGMP by type 5 phosphodiesterases by agents like Viagra. Activation of a membrane-bound G-protein-coupled receptor by PGE

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the corporal smooth muscle cells and activates sGC (FIGURES 1 AND 2). The role of muscarinic receptor-mediated NO release from the endothelium of the corpora cavernosa in erection is controversial, although recent studies suggest a significant role for the contribution of muscarinic receptor activation by ACh released from cholinergic nerve terminals in the penis (50, 73). It has been reported that injection of atropine in a dose of 1 mg/kg iv had no significant effect on the erectile response to pelvic nerve stimulation in the anesthetized dog (22). Dail and colleagues reported a similar observation in a rat model with the administration of atropine and stimulation of the pelvic nerves (31). However, a recent study demonstrated a significant reduction in the erectile response to cavernosal nerve stimulation after administration of atropine (73). In another study, the erectile response to cavernosal nerve stimulation was significantly attenuated at frequencies of 2–16 Hz following administration of atropine 1 mg/kg iv. The erectile response to intracavernous injection of ACh was attenuated, and the response to intracavernosal injection of the NO donor sodium nitroprusside was not altered at the time the response to cavernosal nerve stimulation was significantly attenuated (50). These results suggest that ACh released from cholinergic nerves in response to cavernosal nerve stimulation may mediate ~30% of the erectile response in the rat (50, 73).

It is well established that NO signaling involves an increase in cGMP levels and in cGMP protein kinase 1 activity in penile smooth muscle that leads to vasodilation, increased blood flow, and penile erection (5). NO formation and bioavailability can be impaired by pathophysiological processes and increased oxidative stress (32). Reactive oxygen species (ROS) have been implicated in pathophysiological conditions such as atherosclerosis, diabetes, hypertension, coronary artery disease, and congestive heart failure (80). ROS scavenge NO to form peroxynitrite and consequently impair NO-sGC-cGMP signaling. Oxidative stress involves the generation of the oxidant species superoxide anion and peroxynitrite and has been shown to be capable of oxidizing the normally reduced heme on sGC, rendering the enzyme incapable of responding to NO and generating cGMP (32, 80). Superoxide anion and peroxynitrite have been shown to impair endothelial function and to cause ED (7, 48). Therefore, oxidative stress can induce ED by two mechanisms: 1) reducing NO bioavailability (through generation of superoxide anion or peroxynitrite) or 2) altering the redox state of the intracellular receptor for NO, sGC, by oxidizing its heme iron from a ferrous (Fe$^{2+}$) to a ferric (Fe$^{3+}$) state, which does not bind NO (FIGURE 2) (32). A reduction in oxidative stress has been shown to enhance erectile activity in many animal model ED studies (2, 46, 75). It has been reported that adenoviral-mediated gene therapy enhanced expression of extracellular superoxide dismutase or eNOS and improved erectile function in streptozotocin-treated and aged rats (11, 13, 14, 24). In pathophysiological situations where NO formation or bioavailability is severely depressed, two newly developed classes of agents called sGC stimulators and sGC activators can improve erectile function (FIGURE 2) (70, 78).

CgMP is hydrolyzed by cGMP phosphodiestersases, of which PDE-5 is most abundant (54). The intracellular cGMP concentration is determined by the rate of formation of the cyclic nucleotide by sGC and the rate of inactivation of cGMP by cGMP phosphodiestersases. PDE-5 inhibitors are the first line “gold standard” for treatment of ED, and it has been reported that >50% of ED patients benefit from treatment with PDE-5 inhibitors (9). However, a number of patients do not respond adequately to these oral agents (55). The causes of failure with the PDE-5 inhibitors may include severity of ED, surgical
synthesized by Bayer, including BAY 41-2272, BAY 41-8543, and BAY 60-4552, as well as a sGC-stimulating agent from Abbott Laboratories, A-350619 (FIGURE 2) (10, 50, 58, 63). It was shown that BAY 41-2272 produced weak erectile effects when administered to conscious rabbits intravenously as well as orally by measuring the uncovered penile length (10). However, when BAY 41-2272 was given by either route of administration and followed by an iv dose of the NO donor sodium nitroprusside (SNP), erectile responses were potentiated (10).

BAY 41-8543 is a sGC stimulator that can synergize with NO to increase the catalytic activity of the enzyme up to 200-fold and has recently been shown to induce erection in the rat and to demonstrate synergy with NO in vivo studies (50, 71). In an experimental model of ED produced by chronic cavernosal nerve crush injury, the iv administration of the sGC stimulator BAY 60-4552 and the PDE-5 inhibitor vardenafil produced synergistic beneficial effects on the erectile response to cavernosal nerve stimulation in the rat (63). These results support the concept that combined therapy with a sGC stimulator would improve erectile function in subjects who do not respond adequately to PDE-5 inhibitor therapy alone.

The inhibition of NOS with L-NAME reduces the erectile response to cavernosal nerve stimulation by >90% in the rat (20, 60). The erectile response to the intracavernosal injection of the sGC stimulator BAY 41-8543 is not impaired by NOS inhibition with L-NAME or by cavernosal nerve crush injury, which also reduced the erectile response to cavernosal nerve stimulation by 85% (50). In these studies where NO formation is severely depressed and erectile function is greatly diminished, the intracavernosal injection of BAY 41-8543 can restore normal erectile responses (50). Therefore, sGC stimulators like BAY 41-8543 can be used in the treatment of ED when NO formation or bioavailability is impaired and PDE-5 inhibitor therapy alone is not effective (50, 63) (FIGURE 2).

Although sGC stimulators like BAY 41-8543 can promote a normal erectile response when NO formation is impaired or when erectile function is reduced by pelvic nerve injury, the activity of sGC can also be impaired by extreme oxidative stress (32). Severe oxidative stress can oxidize the iron in the heme binding motif of sGC, which decreases the sensitivity of the enzyme to NO or sGC stimulators (80). In studies with isolated sGC preparations, the enzyme can lose its heme iron under conditions of severe oxidative stress, and the heme-free enzyme can be activated by sGC activators such as BAY 60-2770 (70). In severe erectile dysfunction when sGC is not responsive to NO or sGC stimulators like BAY 41-8543, the heme- and NO-independent sGC activators like BAY 60-2770 can be very effective (FIGURE 2) (70). The sGC activators increase the catalytic activity of sGC when the enzyme is inactivated by oxidative stress or by the loss of its heme iron group (70). The observation that heme-free sGC can be activated by pharmacological agents was first made by Ignarro and Wolin (44). In these studies, it was observed that protoporphyrin IX increased the catalytic activity of heme-free sGC. These studies demonstrated that the heme-free enzyme could be activated to increase the formation of cGMP from procedures that cause nerve damage, hypogonadism, incorrect drug dosage, and/or psychosocial factors (55). Moreover, to be effective, PDE-5 inhibitor therapy requires some basal level of endogenous NO formation in the penile tissues. However, in pathological conditions where NO formation is severely impaired, PDE-5 inhibitors are not effective (49, 52). In addition to oral PDE-5 therapy, other treatments for erectile dysfunction include intrarectal therapy, intracavernosal injections, and penile prostheses (25, 59, 61, 64). Intracavernosal injections have the highest efficacy in patients who do not respond to PDE-5 inhibitor therapy; however, this therapy also has the lowest patient compliance because of the invasiveness of the injection procedure and the high incidence of priapism (61).

sGC Stimulators and Activators

In the treatment of ED when NO formation is severely impaired and when PDE-5 inhibitors are not effective, two new classes of agents have been developed that directly target sGC, increase cGMP formation, and promote penile erections (47, 79). These agents are called sGC stimulators and sGC activators (FIGURE 2) (70, 78). The sGC stimulators are NO-independent, heme-dependent agents that directly activate sGC by binding to a different site than NO, increasing catalytic activity of sGC or normally reduced sGC, which increases cGMP formation and induces erection (32, 39). Inasmuch as sGC stimulators leave the NO binding site on sGC unaffected, sGC stimulators can synergize with even small amounts of NO in catalyzing the conversion of GTP to cGMP (71). The anti-platelet, vasodilating capabilities of the sGC stimulator YC-1 were first described in a 1994 report by Ko and colleagues in which intraperitoneal administration prolonged the tail artery bleeding time in conscious mice (47). YC-1 was the first sGC stimulator tested as an erectile promoting agent in the rat (60). It was shown that YC-1 had erectile activity when given intracavernosally and could also enhance erectile responses to cavernosal nerve stimulation and systemic apomorphine injections (60). From this lead compound, other more potent sGC-stimulating compounds were synthesized by Bayer, including BAY 41-2272, BAY 41-8543, and BAY 60-4552, as well as a sGC-stimulating agent from Abbott Laboratories, A-350619 (FIGURE 2) (10, 50, 58, 63). It was shown that BAY 41-2272 produced weak erectile effects when administered to conscious rabbits intravenously as well as orally by measuring the uncovered penile length (10). However, when BAY 41-2272 was given by either route of administration and followed by an iv dose of the NO donor sodium nitroprusside (SNP), erectile responses were potentiated (10).
GTP and led to the concept that new classes of agents could be effective in targeting an oxidized or heme-free enzyme (33, 44, 72, 79, 80). In experimental animals, sGC can be inhibited and made insensitive to NO by treatment with 1H[1,2,4]oxadiazolo-4,3-aquinoxalin-1-one (ODQ), an agent that has been shown to oxidize the heme iron on the enzyme in many isolated tissue studies and in a few in vivo studies (18, 23, 51, 56, 57, 65, 89). The isolated enzyme studies of Zhao and Marletta showed that ODQ had no effect on basal activity of sGC but blocked the activation of sGC by NO (89). Treatment with ODQ will inhibit the erectile response to NO donors, to cavernosal nerve stimulation, and to sGC stimulators like BAY 41-8543 (50, 51). However, when sGC is inactivated by ODQ, the effect of the sGC activator BAY 60-2770, which has potent erectile activity, will be increased (51a). Moreover, it was demonstrated that BAY 60-2770 could produce a normal erectile response when the cavernosal nerves were injured (51a). Therefore, in extreme pathological conditions when NO formation or bioavailability is impaired and sGC is inactivated and cannot respond to exogenous NO, sGC stimulators, or PDE-5 inhibitors, sGC activators like BAY 60-2770 represent a novel form of therapy than can restore normal penile erectile function. It is therefore possible that sGC activators can promote adequate erectile function in patients with severe ED who do not respond to more traditional therapies. The agents that stimulate or activate sGC can be used alone and in combination with a PDE-5 inhibitor to improve erectile function in patients who do not respond to other forms of therapy.

Rho-Kinase

There are two major mechanisms mediating relaxation of cavernosal smooth muscle. The NO-sGC-cGMP pathway and the adenylate cyclase-cAMP pathway, which is activated by alprostadil (PGE1), which binds to a G-protein-coupled receptor on the membrane of cavernosal smooth muscle, increases the catalytic activity of adenylyl cyclase, and increases intracellular cAMP levels (FIGURE 2). An increase in intracellular cGMP or cAMP concentration activates downstream protein kinases, which act to reduce intracellular calcium concentration, promote cavernosal smooth muscle relaxation, and promote penile erection (FIGURE 1). Another important mechanism in promoting erectile function involves the Rho-kinase pathway, which has a major role in mediating detumescence (12, 28). The importance of this pathway has been demonstrated by observing the pronounced effect of Rho-kinase inhibitors on erectile function (26–28, 30, 67, 69, 82). The effect of the Rho-kinase inhibitor Y-27632 on erectile function was first documented by Chitaley et al. in 2001 and showed that Y-27632 had potent erectile activity in the rat (28). A number of studies have reported potent erectile activity of Rho-kinase inhibitors, which reduce the calcium sensitivity of contractile proteins in cavernosal smooth muscle by increasing myosin phosphatase activity and decreasing the phosphorylation of the myosin light chains promoting corporal smooth muscle relaxation (3, 86). It has been shown that the erectile activity of Rho-kinase inhibitors is NO independent, and it has been suggested that Rho-kinase inhibitors may be useful in the treatment of severe ED, although priapism may occur with the use of longer-acting Rho-kinase inhibitors (28, 51).

Carbon Monoxide

Like NO, carbon monoxide (CO) is a gasotransmitter that is formed in the endothelium. In humans, the endogenous source of CO is from degradation of heme by the heme oxygenases (HO-1, HO-2) to CO, biliverdin, and iron (1). The inducible enzyme HO-1 is upregulated in many pathological conditions, and immunoreactivity studies have shown expression of HO-1 and HO-2 in endothelial cells of the cavernosal artery and the endothelium of the corpora cavernosa (19, 38). CO has been shown to be an effective vasodilator in several blood vessels (74, 84). CO can induce cavernosal smooth muscle relaxation by activating sGC, by opening potassium channels, or by interaction with p450 enzymes (29, 74, 84). Agents that enhance the formation of CO in the corpora cavernosa may have potential use in the treatment of ED in the future.

Hydrogen Sulfide

Hydrogen sulfide (H₂S) is another gas transmitter formed in the endothelium, and the role of H₂S has recently been reviewed by Qiu and colleagues (66). H₂S has been shown to exist as a central and peripheral neurotransmitter (15). H₂S is predominantly produced from the conversion of l-cysteine by the pyridoxal phosphate-dependent enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) and are expressed in a wide range of tissues, including the corpora cavernosa (66, 81). H₂S has been shown to have vasorelaxant properties, and inhibition of H₂S-forming enzymes with DL-propargylglycine (PAG) inhibits the erectile response to cavernosal nerve stimulation in the rat (77, 87). Pharmacological studies have shown that ATP-sensitive K⁺ channel (KᵥATP channel) and the Ca²⁺-dependent K⁺ channel (KᵥCa channel) are the primary mediators of the vasorelaxant response to H₂S (87, 88). However, it has also been shown that...
H₂S can act as a transient receptor potential A1 (TRPA1 ion channel agonist), and it is hypothesized that this activity may be involved in the relaxation of penile smooth muscle (36, 66, 85). The use of agents that act by the H₂S pathway may be of potential benefit in the treatment of ED in the future.

Conclusions: ED, NO, and Therapeutic Potential

NO is the principal mediator of penile erection. A number of studies have demonstrated that a decrease in NO formation and/or bioavailability play an important role in the development of ED, and interventions such as gene therapy, which increase eNOS expression, improve erectile function (24). In the treatment of ED, PDE-5 inhibitors, which decrease the hydrolysis of cGMP, are first-line therapy and are effective in >50% of patients (9). However, when NO formation or bioavailability is decreased below a critical minimum level, PDE-5 inhibitors are not effective, and other forms of therapy are needed. The intraurethral administration of alprostadil (PGE-1), which increases cAMP levels, is effective in patients who do not respond to PDE-5 inhibitors. Other new forms of therapy for ED include the use of stimulators and activators of sGC (FIGURE 2). In severe ED when NO production by the NANC nerves and the corporal endothelium are decreased and when sGC is still responsive to NO and not inactivated or oxidized, the use of a sGC stimulator that increases the catalytic activity of the normally reduced form of sGC and increases cGMP levels in cavernosal smooth muscle will be effective in restoring erectile function. The data in the literature indicate that a sGC stimulator like BAY 41-8543 will produce a normal erectile response when NO formation is inhibited by a NOS inhibitor and muscarinic receptors are blocked with atropine and when the cavernosal nerves have been damaged (50). In the situation where a sGC stimulator is effective, the response to these agents can be enhanced by the addition of a PDE-5 inhibitor. It should be pointed out that clinical trials on the treatment of ED using a combination of the sGC stimulator BAY 60–4552 and a PDE-5 inhibitor have been completed (36a). In the severe pathological situation when sGC is inactivated or oxidized and not responsive to NO or sGC stimulators, only a class of agents called sGC activators like BAY 60–2770 will be effective (FIGURE 2).

Inasmuch, both cAMP and cGMP can reduce intracellular calcium concentration in corporal smooth muscle, and agents that increase cAMP levels are effective in the treatment of ED. PGE-1 and PGE-1 analogs act on a membrane G-protein-coupled receptor to increase adenylyl cyclase activity and increase intracellular cAMP levels and are effective in the treatment of ED (FIGURE 2).

The inhibitors of the Rho-kinase pathway are effective in inducing penile erection (28, 51, 82). These agents can restore erectile function when NO formation is inhibited and the nerves innervating the corpora cavernosa are damaged. The observation that the inhibition of this novel pathway induces penile erection under normal resting conditions can be interpreted to suggest that the Rho-kinase pathway is constitutively active and plays an important role in maintaining the penis in a detumescent state. However, the use of long-acting Rho-kinase inhibitors will cause priapism.

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References


