MINIREVIEW
Role of Nitric Oxide in the Physiology of Erection[1]

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ABSTRACT

Penile erection is understood to be a neurally regulated physiologic event, although the underlying mechanisms have eluded characterization for more than a century. The classical autonomic parasympathetic and sympathetic nervous systems are involved, but the process of erection does not appear to require cholinergic or adrenergic mechanisms. As the search for the principal mediator of penile erection has continued over time, nitric oxide (NO), a gaseous messenger molecule, has been rapidly advanced to fulfill this elusive role. In the recent past, various biochemical and functional data have accumulated to support this understanding. These have shown that NO is an important mediator of corpus cavernosal smooth muscle relaxation and have revealed its potential sources and mechanisms of action in the penis. Additional work involving animal erection models has established that NO mediates physiologic penile erection. This minireview presents these data and includes results from recent clinical trials that have preliminarily evaluated the efficacy of NO-generating or -releasing compounds for the treatment of erectile dysfunction.

INTRODUCTION

Penile erection is a highly regulated physiologic event that comprises increased arterial inflow and restricted venous outflow from the penis, coordinated with corpus cavernosal smooth muscle relaxation [1]. The central nervous system is integral to this process since it receives psychogenic and reflexogenic stimuli and orders these into information that is thereby relayed to the periphery by lumbosacral neuronal pathways. At the local level, nerve fibers, sinusoidal endothelium, and cavernosal smooth musculature of the penis also contribute to the regulation of erection by releasing and processing biochemical substances that determine the erectile tissue response [2].

Contemporary knowledge regarding the physiology of erection includes the understanding that a non-adrenergic, non-cholinergic (NANC) mechanism is principally involved [2, 3]. Diverse mediators have been proposed to account for NANC-mediated responses, including neuropeptides such as vasoactive intestinal peptide (VIP), calcitonin gene-related peptide, and substance P; purines such as adenosine and ATP; decarboxylated amino acids; and other factors including histamine, serotonin, prostaglandins, and bradykinin [4]. However, the definitive agent subserving penile erection has remained elusive [3]. Focus has recently turned to nitric oxide (NO) for this role. This non-obvious substance has now been invoked to execute endothelium-dependent [5, 6] and neurogenically mediated [7, 8] relaxation of vascular and trabecular smooth muscle in the penis.

NO: MESSENGER MOLECULE

A biological role for NO, a labile, potentially toxic gas, has been elucidated only in the recent past. Furchgott and Zawadski [9] originally described endothelium-derived relaxing factor (EDRF), recognizing its apparent endothelial origin in the peripheral vasculature and its ability to induce relaxation of vascular smooth muscle. EDRF was subsequently established by Palmer et al. [10] to be biochemically indistinct from NO. Meanwhile, Ignarro et al. [11] proceeded to show that NO operates in the vascular system by stimulating the formation of cGMP. A potent second messenger molecule similar to cAMP, cGMP is formed catalytically by guanylate cyclase. A critical step is the activation of this enzyme by conformational change following NO binding to the iron atom of its heme moiety [12].

The biological mechanism for cGMP synthesis was better appreciated after it was linked to NO generation from the precursor amino acid, L-arginine, and to the action of NO as a neurotransmitter-like molecule. Following a series of studies showing that brain neurotransmission by various excitatory amino acids is associated with elevated cGMP levels, guanylate cyclase was demonstrated to be activated in neuroblastoma cells by the introduction of L-arginine [13]. Additional experiments involving brain preparations confirmed the association between the formation of NO and cGMP [14]. Cyclic GMP formation was shown by these investigators to be blocked with the application of specific arginine derivatives such as L-N^G-nitroarginine and L-N^G-monomethylarginine, which inhibit NO synthase (NOS). Bredt et al. [15], using immunohistochemical techniques, also localized NOS in selective neuronal populations in the central and peripheral nervous systems. These results taken together contributed significantly to the biochemical and pharmacological basis for NO in biological tissues. NO, it was contended, is released from generator cells, and by lo-

cal diffusion it supposedly interacts with neighboring target cells in which guanylate cyclase is contained. Regarded in this way, NO initiates an unconventional form of signal transduction [16–18]. At present, primary constitutive isoforms of NOS are known to be expressed in endothelial cells and nerves, and their activities require the presence of calcium, oxygen, and NADPH [18].

FUNCTIONAL EVIDENCE FOR NO IN THE PENIS

Recognition of the relaxant effects of NO on smooth musculature, such as that observed early on in the field of gastrointestinal motility [19–22], prompted research efforts that next characterized this mechanism in the erectile tissue of the penis. Several functional studies involving isolated corporal tissue specimens obtained from diverse species, including the human, suggested a role for NO as a mediator of cavernosal smooth muscle relaxation [16, 23–27]. In these elegant organ bath experiments, direct application of NO or NO substrates caused tissue relaxation resembling that achieved with electrical field relaxation. The relaxation was susceptible to axonal conduction blockade as is seen to occur with application of tetrodotoxin; this affirmed the neurogenic basis for the response. The implication of NANC neurotransmission was met, since these effects were resistant to cholinergic receptor blockade and occurred after selective adrenergic nerve inhibition. However, with the application of inhibitors of NO synthesis, the effects of electrical field stimulation were abolished. The degree of the effect on corporal tissue was concentration-dependent and obeyed the relative pharmacologic potencies of specific inhibitors. Similarly, NANC-mediated relaxation was shown to be blocked by the inhibition of cGMP formation that occurs with the infusion of methylene blue, whereas it was facilitated with inhibitors of cGMP phosphodiesterase [28, 29].

The next approach in the study of NO in penile erection was pursued by several investigators who similarly questioned the importance of this substance in vivo. In the rat [8, 30, 31] and rabbit [32], electrically induced erections were inhibited by i.v. and intracavernous administration of inhibitors of NO synthesis, whereas NO or NO substrates reversed these effects. In dogs, tumescence consistent with that achieved by pelvic nerve stimulation was achieved with intracavernous injection of NO-releasing substances including nitroprusside and S-nitroso-N-acetylpenicillamine, whereas the opposite effect resulted from the intracavernous administration of a NOS inhibitor or methylene blue [33, 34]. Conversely, the intracavernous injection of cGMP or a specific cGMP phosphodiesterase inhibitor was shown to enhance erectile responses. The in vivo erectile effects of intracavernosally injected NO-mediating agents were also demonstrated in cats with NO-releasing agents (S-nitroso-cysteine and S-nitroso-N-acetylpenicillamine), whereas these effects were attenuated with direct NOS inhibition [35]. Similar studies involving monkeys have also shown that the NO-cGMP signal transduction system operates in the primate model to mediate penile erection [36, 37].

SOURCES OF NO IN THE PENIS

During the time that NO had come to be established as a major, important regulator of physiologic penile erection, other investigations were being carried out to identify the source of NO in the penis and to delineate the mechanisms involved in its activity. Gillespie et al. [38] had earlier advanced the hypothesis that NO may operate in the urogenital system as a neuronal, transmitter-like messenger. Localization studies provided one direct approach for testing whether NO might function as a neurotransmitter in the penis, as had been done previously in neuronal tissues. Using a biochemical assay for the catalytic activity of NOS, Burnett et al. [8] established particularly high amounts of NOS in the penis, membranous urethra, and major pelvic ganglion among various pelvic structures of the male rat. Immunoblot studies confirmed the neuronal isoform of NOS in these structures [8].

Precise localizations of NOS in the pelvis and genital structures were established through the use of immuno-histochemical and enzyme histochemical methods. In the rat [8, 39–42], dog [8], bull [43], and human [44], neuronal NOS was localized to the pelvic plexus, to the cavernous nerves and their terminal endings in the cavernous tissue, to the dorsal penile nerves, and to nerve plexuses in the adventitia of dorsal and deep cavernosal penile arteries and their tributaries, the helicine arteries. Bilateral cavernous nerve transection in rats abolished immunohistochemical staining in NOS-containing penile neurons, whereas staining persisted in vascular endothelium, in which the antibody had cross-reacted to endothelial NOS [8]. It was inferred from these data that NO is produced by nerves acting as a post-ganglionic neurotransmitter and that it is released through efferent neuronal pathways in response to erogenic stimuli. In concept, NO locally diffuses to vascular and trabecular smooth musculature in the penis, thereby stimulating both vasodilation and tissue relaxation; therefore, blood flow to the penis increases and erectile tumescence occurs.

Immunohistochemical and axonal tracing methods have been used to further examine the distribution of NOS in neuronal pathways to the pelvic viscera including the penis [42, 45, 46]. Preganglionic autonomic neurons in the pelvic ganglia of the rat have been shown to contain NOS, precise origins having been identified within the lumbosacral spinal cord. In addition, NOS immunoreactivity predominates among urogenital organs in efferent pathways to the penis and urethra. These data importantly suggest the selective modulatory role of NO in pelvic neurotransmission, and they advance the concept that this substance mediates penile erection at a second order of neuronal regulation as well.

Despite the neuronal basis for NO in NANC-mediated penile erection, additional sources of NO have been investigated. A constitutive isoform for NOS includes the endothelial variety, biochemically distinct from the neuronal isoform [47]. Ample evidence suggests that the endothelium in the penis liberates NO under acetylcholine stimulation [48]. However, since the removal of sinusoidal endothelium from corporal tissue in in vitro experiments did not eliminate relaxant effects [7, 16], it is probable that the endothelium truly suffices as an auxiliary source of NO in the penis. Whether the smooth musculature provides a source of NO at baseline conditions is controversial. It is likely that an inducible isoform of NOS exists in smooth muscle cells and is typically expressed with the introduction of cytokines or endotoxin [49]. However, smooth musculature remains a possible source liberating NO under normal physiologic conditions, as recently shown for gastrointestinal peristalsis [50].

NO MECHANISMS IN THE PENIS

The vasodilatory properties of organic nitrates and nitrites are well recognized by their relaxant effects on vascular smooth muscle [4]. Several investigations have demonstrated the potential therapeutic role for nitrovasodilators for erectile dysfunction, based on the premise that the erectile response requires dilatation of the arterial vessels and relaxation of the smooth muscle in sinusoids [1, 51, 52]. The results of these early investigations can be supported biochemically. The smooth muscle relaxation attributed to NO, or a NO-releasing vasodilator, involves the activation of soluble guanylate cyclase that catalyzes the production of a second messenger, intracellular cGMP. Several mechanisms are proposed that would account for the smooth muscle relaxant activity resulting from an NO-induced increase in intracellular cGMP [4, 12]. These include 1) inhibition of generation of inositol triphosphate; 2) increased sequestration of cytosolic Ca^{2+}; 3) dephosphorylation of the light chain of myosin; 4) inhibition of Ca^{2+} influx; 5) activation of protein kinases; 6) stimulation of membrane Ca^{2+}-AT-Pase; 7) opening of K^+ channels; and 8) inhibition of a cAMP phosphodiesterase. The precise mechanisms that operate in corpus cavernosal smooth muscle remain unclear, although it can be anticipated that these will be elucidated with ongoing studies. An alternative possibility whereby NO may facilitate erection is via enhancement of penile blood flow and engorgement resulting from a cGMP-induced decrease in platelet aggregation and adhesion [17, 49].

The role of NO in the physiology of erection may be influenced by a host of modulatory factors. Local oxidative conditions and reacting substances may affect the action of NO. These include oxyhemoglobin, intracavernous oxygen tension, intracellular Ca^{2+} stores, superoxide anion, and intracavernous pH [4, 7]. Advanced glycosylation end products exert an antiproliferative effect on NO by directly in-

activating the molecule [53], and it is this mechanism that is postulated to be altered in impaired endothelium-mediated relaxation of corpus cavernosal tissue in diabetes mellitus [54]. Androgen ablation increases NANC nerve-mediated corporal relaxation by some reports [55], whereas others suggest that this exact condition results in attenuated erectile mechanisms governed by NO [30]. The mechanism of androgen action remains unclear, although it is possible that the activity of NOS can be hormonally regulated via its known regulatory sites [56].

Major interest continues to be given to the contributions and possible interactions of various neuronal systems and NO-dependent pathways on erectile physiology. The leading candidate for co-transmission is VIP, which may account for a NO-independent NANC-mediated regulatory pathway in the penis. There exist various data indicating the significance of VIP in penile erection. VIP-immunoreactive neuronal fibers course in trabecular smooth muscle of the penis, and VIP has produced erectile responses both in vitro and in vivo [57, 58]. In several neuronal systems, the coexistence of NO and VIP has been determined immunohistochemically and functionally, implying their synergistic action [15, 59, 60]. In the gastrointestinal tract, VIP induces NO synthesis in smooth musculature [50]. Direct neurostimulation of endothelium by acetylcholine represents an alternative mechanism for NO release in the penis [25, 48]. Substance P and bradykinin may also behave in the penis by stimulating NOS formation of NO [5, 35].

NO IN THE MANAGEMENT OF ERECTILE DYSFUNCTION

From a clinical perspective, an improved knowledge of the physiology of erection and the influence exerted by NO on this process can be expected to lead to management strategies for disorders of erectile function. Stief et al. [61] preliminarily demonstrated the therapeutic utility of NO in restoring erectile function in impotent men with the intracavernous administration of linsidomine chlorhydrate (SIN-1), a pharmaceutical agent that releases NO nonenzymatically. However, Porst [62] compared intracavernosally injected SIN-1 with prostaglandin E1 in men with erectile dysfunction and found that SIN-1 elicited an inferior erectile response. Wegner and Knispel [63] also found that intracavernosal injections of SIN-1 were no more effective than injection of prostaglandin E1 in men whose erectile impairment was attributed to venous occlusive dysfunction. These investigators suggested that NO release may be predominantly involved in the "arteriogenic" or "neurogenic" aspects of normal erectile function. It is apparent that the therapeutic advantages of NO remain to be exploited. A major constraint is that NO is a labile, short-lived gaseous molecule that is biologically active in a variety of tissues [17]. Therefore, potential treatments must arrive at an ideal stable compound that efficiently releases or generates NO following its discrete delivery to the cavernosal smooth mus-

culature. NO donor agents that are currently available and known NO intermediates such as S-nitrosothiols probably represent merely the first line of pharmaceutical alternatives yet to come.

Diagnostic implications for the role of NO in penile erection have also been explored. Positive histochemical staining for NOS in human cavernosal tissue biopsies is consistent with a history of cavernous nerve integrity and can be offered as a diagnostic tool for neurogenic impotence [64]. On the other hand, the measurement of NO metabolites either in peripheral or cavernosal blood bears no association with erectile status and may not suffice for diagnostic purposes [65].

OVERVIEW

In recent years, the physiology of erection has been rapidly advanced. This understanding has evolved from a profound recognition of the neuroanatomic principles of erection and an improved knowledge of the physiologic mechanisms that regulate erectile function. Considerable data have emerged indicating that NO acts as a principal mediator of penile erection. Initial experiments established the requirement for NO in the relaxation of vascular and trabecular smooth muscle in the penis, and this was followed by additional in vivo studies establishing NO in physiologic penile erection. Penile nerves primarily produce and release this product, which diffuses locally and stimulates smooth muscle relaxation via a cGMP-induced mechanism. Potential contributing sources of NO in the penis include the sinusoidal endothelium and the corporal smooth musculature itself. These different sources imply that diverse mechanisms may operate to generate NO. Various local substances may also interact with NO, modulating its effects in the penis. Ongoing laboratory and clinical investigations can be expected to further characterize and advance NO in the mediation of penile erection. The future holds promise for clinical applications resulting from these investigations.

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