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Invited review

Neurophysiological basis of penile erection[1]

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Abstract

Penile erection involves a complex interaction between the central nervous system and local factors. It is a neurovascular event modulated by psychological and hormonal factors. The discovery of nitric oxide (NO) as an intercellular messenger or neurotransmitter paved the way for identifying important mechanisms underlying physiological and pathophysiological events in the penis, in addition to providing the knowledge for the development of new therapeutics based on a novel concept of molecule and cell interaction. Despite the fact that sinusoidal endothelial cells also produce and release NO in response to chemical and possibly physical stimuli, roles of neurogenic NO in penile erection appear to be more attractive and convincing, since the pharmacological neuromodulation represents an essential step to attaining penile erection. Erectile dysfunction (ED) is caused by a variety of pathogenic factors, particularly impaired formation and action of NO. Hence, a thorough knowledge of the physiology of erection is essential for future pharmacological innovations in the field of male ED, particularly targeting NO or intracellular cyclic GMP, which represent the most promising therapeutic approach to treat patients with ED.

Introduction

Penile erection is a neurovascular event modulated by psychological and hormonal factors. This brief review will describe the functional response for erection with special emphasis on neural components. The topic of erectile dysfunction will be considered and finally, the recent advances in the treatment of erectile dysfunction will be discussed.

Physiology of penile erection Penile erection involves a complex interaction between the central nervous system and local factors. The penis is innervated by autonomic (sympathetic and parasympathetic nerves) and somatic nerve fibers. Overall, erection is a neurovascular event modulated by psychological and hormonal factors. Upon sexual stimulation, neurotransmitters are released from the cavernous nerve terminals and also vasoactive relaxing factors from the endothelial cells of the penis, which relax arteries and arterioles supplying the erectile tissue, increasing the penile blood flow. Concomitantly, relaxation of the trabecular smooth muscle increases the compliance of the sinusoids, resulting in an engorgement of the penis with blood. Therefore, penile erection takes place when both dilation of the penile arteries and relaxation of the erectile tissue occur. Because the erectile tissue is surrounded by the tunica albuginea, a tissue that does not distend easily, the increased blood flow to the penis increases not only the penile volume but also intrapenile pressure. This distension causes mechanical compression of the emissary veins, which impedes their ability to drain blood and thereby results in penile rigidity. Detumescence is the result of a cessation of neurotransmitter release, the breakdown of second messengers or sympathetic discharge during ejaculation. Contraction of the trabecular smooth muscle restores the venous outflow, the trapped blood is expelled, and flaccidity returns[1].

Peripheral regulation of penile erection The nerves and endothelium of sinusoids and vessels in the penis produce and release transmitters and modulators, which interact in their control of the contractile state of the penile smooth muscles. The different structures of the penis are functionally regulated by efferent sympathetic and parasympathetic

nerves, and the major neurotransmitters in postganglionic fibers are norepinephrine and acetyl-choline, respectively. Sympathetic input is antierectile, whereas parasympathetic and somatic input are proerectile. Both sympathetic and parasympathetic fibers reach the pelvic or inferior hypogastric plexus where autonomic input to the penis is integrated; the cavernous nerves originate from this plexus, and innervate the helicine arteries and erectile tissue. Intracavernous nerves are encased in fibrous tissue, which prevents their compression during an erection. The dorsal penile nerves, branches of the pudendal nerves, and the ilioinguinal nerve also innervate the penis. These nerves provide sensory input from the glans penis and skin, and penile root[2].

About a decade ago, several investigators provided evidence for functional roles of nonadrenergic noncholinergic (NANC) inhibitory and excitatory nerves, containing transmitters and transmitter/modulator-generating enzymes, such as nitric oxide synthase (NOS) and heme oxygenases (HO). NANC transmitters/modulators may be found in adrenergic and cholinergic nerves[3], which should make it more meaningful to define nerve populations based on their transmitter content. Although various polypeptides have been regarded as inhibitory neurotransmitters[3-5], the discovery that nitric oxide (NO) functions as a mediator synthesized in and released from the vascular endothelium[6,7] and as a neurotransmitter in inhibitory nerves innervating the penis represented a breakthrough in the comprehension of the neurophysiological basis of erection. Figure 1 demonstrates the experimental protocols for establishing the role of NO as a neurotransmitter in the erectile response of the rat penis.

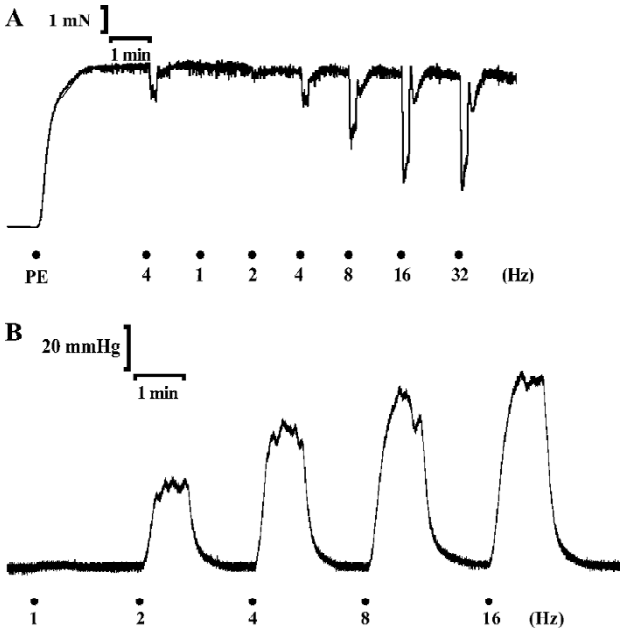

Figure 1. (A) typical responses to electrical field stimulation (1–32 Hz for 10 s; 20 V) of a rat corpus cavernosum strip contracted with phenylephrine (PE, 10 μmol/L). Neurogenic relaxation was obtained in strips pretreated with bretylium tosilate (30 μmol/L) and atropine (1 μmol/L) to block adrenergic and cholinergic transmission, respectively. (B) Representative tracing showing the *in vivo* erectile response, measured as increases in intracavernous pressure, following electrical stimulation of the cavernous nerve (1–16 Hz for 1 min; 5 V) in anaesthetized rats. Dots below the tracings represent the application of electrical stimulation. These relaxation responses in the isolated strip and the erectile responses in the intact animal are blocked largely by NO synthase inhibitors. These observations are consistent with the concept that NO acts as a neurotransmitter in NANC nerves innervating the penis.

Erectile function and nitric oxide

Synthesis of NO and the consequences of NO binding to soluble guanylyl cyclase is essential for the erectile process. Identification of NO to be a neurotransmitter has been achieved by the use of NOS inhibitors in the corpus cavernosum of the penis[8]. NO, an inorganic and labile molecule, is liberated immediately upon synthesis by neuronal NOS (nNOS) from substrate *L*-arginine. To date, it is widely accepted that NO is the main neurotransmitter mediating penile erection, which is released during NANC neurotransmission. Upon its release, NO diffuses locally into adjacent smooth muscle cells of the corpus cavernosum and binds with its physiologic receptor, soluble guanylyl cyclase[9]. The enzyme becomes activated following this interaction whereupon the enzyme catalyzes the conversion of guanosine triphosphate (GTP) to 3',5'-cyclic guanosine monophosphate (cGMP). This cyclic nucleotide then serves as a second-messenger function by activating protein kinase G, alternatively known as cGMP-dependent protein kinase I (cGKI), which in turn exerts actions involving ion channels and contractile regulatory proteins that regulate the contractile state of corporal smooth muscle. The consequence is the decay in cytosolic calcium concentration and relaxation of the smooth muscle, resulting in dilation of arterial vessels and increased blood flow into the sinuses of the corpora cavernosa[1,10]. Thus, at the onset of sexual stimulation, neuronal NO induced by neuronal depolarization and endothelial NO largely generated in response to shear forces brought on by increased blood flow in the penis serve, respectively, as a neurotransmitter initiating the erectile process and as a paracrine factor sustaining the full physiologic response. On the other hand, phosphodiesterase-5 (PDE5) operates in this signal transduction pathway to restrain erec-

tile effects. This enzyme is predominantly expressed in the corpus cavernosum[11] and functions as a cGMP-specific phosphodiesterase, which catalyzes the hydrolysis of cGMP to GMP[12,13]. Accordingly, in the penis, the enzyme controls cGMP accumulation caused by NO signaling and consequently limits its relaxant actions.

Erectile dysfunction

Erectile dysfunction (ED) is defined as the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance[14]. ED is highly prevalent and by current estimates, 30 million men in the US and 150 million men worldwide are affected[15] and occurs in 19%–64% of men aged 40–80 years, both in developing and industrialized countries. Emotional, physical, and medical factors contribute to ED, and this condition may also be a symptom of various chronic diseases. ED may affect total health, relationships, and overall quality of life[16,17]. Organic causes are now understood to constitute more than 80% of clinical presentations. Associations include diabetes mellitus, cardiovascular disease, hyperlipidemia, cigarette smoking and obesity, indicating their significance as a public health problem. Furthermore, the disorder is correlated with anxiety, depression, interpersonal relationship difficulties and even violence[18].

The exact etiological mechanisms responsible for abnormal erectile response have yet to be determined. However, organic and psychogenic factors may cause alterations in the NO/cGMP pathway and impair smooth muscle relaxation and/or increase smooth muscle contraction, thereby resulting in ED. Ultimately, the treatment of ED has been revolutionized from only surgical options (penile prostheses or revascularization) to intracavernosal and intraurethral administered agents [eg, prostaglandin E1 (PGE1), papaverine, phentolamine] that paved the way to an effective oral therapy such as PDE5 inhibitors. The clinical efficacy of oral agents such as apomorphine, phentolamine, sildenafil, tadalafil, and vardenafil represent the beginnings of noninvasive pharmacological treatment for ED.

It is widely known that ED is associated with diseases reported to be related with decreased NO bioavailability such as arterial hypertension, hypercholesterolemia and diabetes. However, a recent study showed that ED appears in spontaneously hypertensive rats before they become hypertensive, suggesting that ED might be a marker for hypertension[19]. Also, it was recently demonstrated an impairment in both endothelium-dependent and -independent dilation in patients with ED, who did not present diseases such as coronary artery disease or diabetes mellitus. It was suggested that ED is associated with an abnormal function in the NO-cGMP pathway even in the absence of any apparent cardiovascular or metabolic disease[20]. Besides its direct effects in the cavernosal smooth muscle, NO also contributes to the maintenance of the erectile state by inhibiting contractile mechanisms involving noradrenaline release, reactive oxygen species (ROS) formation and Rho-kinase activity, thus favoring the corpus cavernosum relaxation.

Treatment of erectile dysfunction

In the last decade, even with the introduction of orally-administrated PDE5 inhibitors, the search for new drugs for the treatment of erectile dysfunction has been extensive. Since the discovery of NO as the main neurotransmitter mediating penile erection, several studies regarding the role of NO/cGMP pathway in the erectile function have been performed. Both the endothelium and the NANC nerves of the corpus cavernosum serve as the source of NO, and thus, more than one isoform of NOS is involved. Several investigators have demonstrated the presence of nNOS in the cavernous nerves and their terminal endings within the corpora cavernosa, as well as in the branches of the dorsal penile nerves and nerve plexuses in the adventitia of the deep cavernous arteries[9,21–26]. In human corpus cavernosum, nNOS is present in nerve fibers innervating the cavernous body and cavernosal arteries whereas eNOS is largely found in the endothelial cells covering the cavernous spaces and helicine arteries but not in the trabecular smooth muscle cells[27]. Given the importance of NOS in the generation of NO, NOS activity and expression represents an important factor to be investigated. It is known that the activity of constitutive NOS is completely dependent on calcium, calmodulin and NADPH and addition of tetrahydrobiopterin (BH_4) increases NOS activity by approximately 30%[28]. Furthermore, it was recently demonstrated that the BH_4, applied systemi-cally, improves erectile function[29], suggesting that cofactors for NOS also might be important targets in the treatment of erectile dysfunction.

Similarly, several studies have shown that ROS generation decreases NO bioavailability, impairing the erectile function[30]. However, in the corpus cavernosum of streptozotocin-induced diabetic mice, vitamin E and sodium selenate partially reversed the endothelial dysfunction and the impairment of neurogenic relaxation[31]. Similarly, ascorbic acid also prevented the impaired relaxation of acetylcholine observed in middle aged non-diabetic and diabetic rats[32], suggesting that decreased oxidative stress increases NO bioavailability improving the erectile function. Also, physical training is shown to reduce ROS generation and to raise

NOS gene expression and activity. Indeed, neurogenic relaxation elicited by electrical field stimulation as well as the relaxant response evoked by exogenous NO were increased in rats submitted to endurance training, demonstrating that physical training improves the NO/cGMP signaling pathway, and thus the erectile response[33,34]. Taken together, these data show an improvement of erectile function likely related to a decreased ROS generation.

Since the primary synthesis of cGMP, driven by NO production and release during sexual arousal, is a key to the mechanism for erection, other means to achieve an enhancement of NO responses is represented by the use of PDE5 inhibitors, such as sildenafil, vardenafil and tadalafil. These drugs target PDE5 and inhibit the hydrolysis of cGMP, thus preserving cGMP and permitting the cyclic nucleotide to activate cGKI to a greater extent than at baseline conditions such that corporal smooth muscle relaxation is enhanced. Their precise mode of action is to bind to the catalytic domain of PDE5 blocking substrate degradation. However, the efficacy of the PDE5 inhibitor, sildenafil, in the relaxation of the corpus cavernosum is decreased when NOS is blocked[35]. Recently, a novel NO-donating derivative of sildenafil, NCX 911, was developed and showed to improve the relaxation induced by carbachol and decrease the superoxide formation compared to sildenafil citrate, in the corpus cavernosum of hypercholesterolemic rabbits[36]. Also, it was demonstrated that the potency of this compound in the corpus cavernosum is not altered when the synthesis of NO is inhibited by *L*-NAME[35], suggesting that a combination of a NO-releasing compound with a PDE5 inhibitor, might be a more interesting tool for the treatment of ED.

Another pathway that has been associated to ED and extensively investigated is the RhoA/Rho-kinase pathway, which mediates Ca^{2+} sensitization in the penile circulation and maintains the penis in the flaccid state. Indeed, it was recently demonstrated that intraperitoneal administration of H-1152, a Rho-kinase inhibitor, enhanced the erectile response produced by stimulation of the cavernous nerve[37]. Futhermore, the ED observed in aged rats or in the vasculogenic model of ED is likely associated to an increased RhoA/Rho-kinase pathway activity[38–40]. Further, it was shown that the chronic treatment with fasudil, a Rho-kinase inhibitor administrated orally, prevented the impaired erectile function by reversing the increased RhoA/Rho-kinase activity seen in vasculogenic model of ED[40]. Similarly, the impaired corpus cavernosum pressure observed in castrate model of ED, was restored by inhibiting this pathway[41]. Taken together, these data show that the RhoA/Rho-kinase pathway interferes with the NO/cGMP pathway and also might represent an important target in the treatment of the erectile dysfunction.

All of these observations indicate that, although several studies have been searching new approaches for the treatment of ED, it seems that increase in NO bioavailability and consequent improvement of the corpus cavernosum relaxation, still represents the main target for the treatment of ED.

Concluding remarks

There is a broad range of evidence indicating that NANC neurotransmission has a vital role in mediating penile erection via a NO/cGMP mechanism. Continuous advances in our understanding of the physiology of penile erection should help to elucidate further the mechanisms involved in the pathophysiology of ED, and ultimately define alternate therapeutic strategies to preserve this signaling pathway.

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