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# PnPP-19, A Spider Toxin Derivative: New Hope for the Treatment of Sexual Dysfunction?

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## Abstract

The incidence of erectile dysfunction (ED) is increasing every year worldwide, and affects primarily those patients that suffer from vascular diseases, such as diabetes and hypertension. There is an increasing number of patients for whom first-line and second-line pharmacotherapy are not indicated. Animal venoms and toxins have been envisaged as pharmacological tools that enable elucidating the mechanisms that lead to erectile function, as well as give hints for drug development to treat ED. Recent studies have demonstrated the promising action of the synthetic peptide PnPP-19, derived from a spider toxin (PnTx2-6), on erectile function. This peptide potentiates erection at 4 and 8 Hz *in vivo* and *ex vivo*. It shows no apparent toxicity and presents low immunogenicity to mice. It does not affect sodium channels or rat hearts. Therefore, PnPP-19 may emerge as a new pharmacological tool and shows favorable properties as a bioactive molecule in the treatment of ED.

**Keywords:** PnPP-19; Erectile function; PnTx2-6

## Introduction

Historically, ED was first treated through intra-cavernosal/intra-urethral administration of vasoactive drugs or through surgical interventions. The discovery of nitric oxide (NO), in the late 20th century, as the main actor in corpus cavernous smooth muscle relaxation and the most important peripheral neurotransmitter in erectile function, led to the development of drugs that interfered in this mechanism. Among them, phosphodiesterase-5-inhibitors (PDE5I), which could be orally administrated, revolutionized the sexual medicine field [1,2]. Today, first-line therapy for the treatment of ED comprise orally and sublingually administered drugs, while intra-cavernosal injection therapy is considered only as a second-line therapy [3].

## Erectile Dysfunction

Erectile dysfunction (ED) adversely affects the life of millions of men, and is the most commonly treated sexual disorder today. ED is defined as a persistent inability to maintain or achieve a penile erection that allows sexual intercourse [4]. This comorbidity is commonly a result of the interaction of various physiologic disorders, which can have vasculogenic, neurogenic or endocrinologic basis. In addition, ED can be induced by medication or substance consumption, poor health, cardiovascular disorder, or physical disorders, as penile rupture [5]. Psychogenic causes, related to trauma from previous sexual experiences and low self-confidence may contribute to ED [6]. Patients with cardiovascular risk factors (hypertension, diabetes, hyperlipidemia, sedentariness, tabagism) and vascular abnormalities for penile blood supply present high incidence of ED [7]. According to a recent study, more than 30% of the European men surveyed, at the age of 60 or older, were affected by ED [8]. In the USA, 18.4% of adult men reported ED and, in Brazil, the prevalence of ED was 45.1% [9,10].

The erectile process has been extensively investigated, leading to the elucidation of many of the complex molecular pathways involved [6]. These findings have enabled the design and development of drugs that target various aspects of this complex process.

## Current Treatments for Erectile Dysfunction

Although PDE5I has been widely used, there is a growing need for alternative pharmacotherapy for the treatment of ED, as a result of the rise in the number of elderly people, and the recognition that a great number of ED patients do not respond to PDE5I [11].

## Venoms and Toxins Active on Erectile Function: New Perspectives for the Treatment of ED

The earliest reports on erectile dysfunction treatment that are found in ancient medical literature prescribe the use of herbals and natural ingredients. Nowadays, many herbal supplements claim therapeutic effects in male sexual dysfunction [12,13]. Additionally, other substances that have been scientifically investigated over the last 20 years comprise toxins from animal venoms, mainly from scorpions and spiders [14].

Arthropod venoms have attracted attention for the development of new therapeutic drug models. Interestingly, some animal venom peptides, especially from spiders and scorpions, can cause priapism (a persistent and painful penile erection not dependent on sexual stimulation) [15]. Many studies using experimental models have shown the ability of these peptides to promote priapism and/or erection, envisaging the development of bioactive molecules to treat and/or study ED [14,16]. These molecules evoke complex effects on ion channels, chiefly sodium, potassium and calcium channels [17-19]. For

example, crude *Tityus serrulatus* venom and some of its purified toxins cause cavernosal smooth muscle relaxation *in vitro*, using rabbit and human samples [20,21]. Similarly, *Androctonus australis* and *Buthotus judaicus* scorpion venoms also relax cavernosal tissue [22].

Spider venoms and some of their purified proteins, peptides or biologically active molecules selectively target a variety of vital physiological functions, being described in the development of drugs for various applications [19,23-25]. For example, *Phoneutria nigriventer* crude venom contains potent excitatory neurotoxins [19,26,27]. Among these toxins, PnTx2-5 and PnTx2-6, which are 89% similar in their primary structure, also renamed δ-CNTX-Pn1b and δ-CNTX-Pn1c, respectively, were identified as directly responsible for priapism [25,28]. Since then, many studies have been published trying to solve the mode of action of these toxins in erection. Both toxins were described as targeting site 3 from sodium channels, slowing down the sodium current [29]. The mode of action through which these spider and scorpion toxins induce priapism in rats and mice involves the nitric oxide pathway. The involvement of nitric oxide synthase (NOS) was elucidated by using a nonselective NOS inhibitor, N(omega)-nitro-L-arginine methyl ester (L-NAME), which decreased the effect of the toxins on priapism [14,25,28].

Upon sexual stimulation, NO is released and induces relaxation of the penile smooth muscle, increases blood flow to the penile region and augments intracavernosal pressure, leading to penile erection. The NO released from penile endothelial cells or nitrergic nerves is the main mediator involved in erectile function, and PnTx2-6 injection was shown to increase NO release in corpus cavernous tissue [25]. After PnTx2-6 treatment, a microarray study using mice penile tissue showed differential expression of 10.4% of the genes involved in the NO pathway, shedding light to the effect of this toxin on the NO pathway [30]. In addition, PnTx2-5, when intra-peritoneally injected in male mice, caused penile erection and hypersalivation, severe respiratory distress and death. L-NAME partially prevented these effects and nNOS-selective inhibitor 7-Nitroindazole completely abolished them, suggesting that nNOS is the major player in the induction of erection by these toxins [19,28]. Both toxins, PhTx2-5 and PhTx2-6, are interesting pharmacological tools to study ED. PnTx2-5 has been less investigated compared to PnTx2-6, but data suggest that this toxin could also be involved in penile neuronal depolarization [28]. The mechanism of action whereby PnTx2-5 and PnTx2-6 promote cavernosal relaxation and enhance erectile function is not completely elucidated. However, the results strongly suggest that it is via NO/Cyclic Guanosine Monophosphate (cGMP) pathway [28,31].

Experiments on cavernosal strips using the pharmacological inhibitor μ- conotoxin GVIA (N-type calcium channel blocker) and knockout mice (nNOS-/-, eNOS-/-) suggested that the relaxation promoted by PnTx2-6 depends on N-type $Ca^{2+}$ channels in nitrergic nerve endings, which are the main source of NO release during erection [31,32]. Thus, taking into account the action of PnTx2-6 on sodium channels, slowing down their inactivation and maintaining cell depolarization, this condition has been suggested to promote the entrance of a higher amount of $Ca^{2+}$ into the cell, which activates nNOS and consequently increases NO availability, leading to penile erection. Additionally, the cavernosal relaxation evoked by PnTx2-6 is independent on phosphodiesterase 5 (PDE5) inhibition and this toxin restores the erectile function in hypertensive (DOCA Salt), diabetic, and elderly rats, in different proportions and prevents muscle atrophy in a rat model [25,31,33,34].

However, PnTx2-6 shows dose-dependent side effects, such as intense vascular congestion in kidney, liver, lungs and myocardium, discrete brain edema [35] and prolonged pain (unpublished data). Furthermore, obtaining large amounts of PnTx2-6 is challenging and its chemical synthesis is complex, because it contains 5 disulfide bridges. Its expression in heterologous systems is possible but costly [36,37].

## The Promising PnPP-19 Peptide

PnPP-19 is a short peptide (19 amino acids) designed *in silico* from the tertiary structure of PnTx2-6 and represents a discontinuous primary structure of the epitope of this toxin. PnPP-19 supposedly represents the active core of the PnTx2- 6, bearing the amino acids described as responsible for the specific interaction of the native toxin with site 3 of sodium channels [29,37]. A molecule with a shorter sequence than PnTx2-6 is expected to bring many advantages: higher specificity, lower toxicity, lower immunogenicity, lower cost and simpler synthesis. PnPP-19 peptide can be easily synthesized and, similarly to the native toxin PnTx2–6 at the same molar doses, potentiates erectile function at low frequencies *in vivo* (anesthetized rats) and *ex vivo* (cavernous strips) [37]. To illustrate this effect, Figure 1 shows a representative graph of the effect of PnPP-19 (10 nM) on rat corpus cavernous strips. Surprisingly, it does not act on any sodium channel subtypes that have been tested [37].

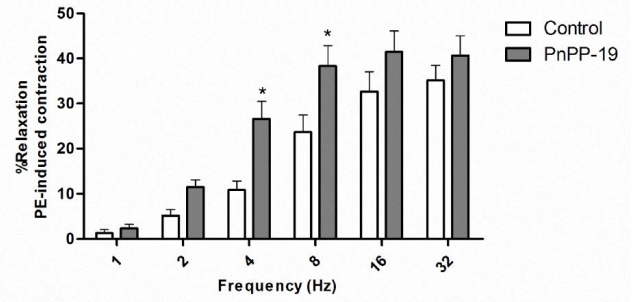


**Figure 1:** PnPP-19 improves relaxation (i.e., erectile function) in cavernosal strips. Five isolated cavernosal strips were pre-contracted with phenylephrine ($10^{-5}$ M) and after EFS-mediated relaxation. Cavernosal tissue incubated for 10 min with PnPP-19 ($10^{-8}$ M) showed significant enhance in cavernosal relaxation at low frequencies (4 and 8 Hz). Asterisk indicates PnPP-19 vs control 2-way ANOVA plus Bonferroni post test ($p < 0.05$).

According to the literature, the use of PDE5Is for the treatment of ED is a very attractive and effective therapy, with few side effects. However, PDE5I therapy does have limitations, especially for patients with active coronary ischemia, congestive heart failure, borderline low blood volume and pressure status, patients with multidrug antihypertensive therapy regimens and those who may need organic nitrates for the treatment of angina [7]. In addition, its effect requires intact NO relaxing nerve fibers and healthy cavernous endothelium, what impairs 30% to 35% of patients from responding to the treatment, stimulating the search for alternative drugs [38,39]. Although PnTx2- 6 effectively potentiates erectile function *in vivo*, this toxin acts on isolated rat heart and slows down sodium currents in rat cardiomyocytes, besides acting on other sodium channel-subtypes

distributed in other organs. In contrast, PnPP-19 does not show effect on rat cardiomyocytes or on isolated heart, even at high concentrations (37.8 nM to 37.8 μM) [37].

The NO relaxation response elicited by PnPP-19 is mainly dependent upon cGMP, synthesized by soluble Guanylyl Cyclase (sGC). Deficiency in NO/cGMP erection pathway is associated with many diseases. PnPP-19 increased cGMP levels in cavernous preparations in the presence of EFS (8 Hz). This effect was inhibited by L-NAME and partially prevented by 7-NI, suggesting that it partly depends on neuronal NOS activity [37]. The authors believe that it is possible that other types of NOS, besides neuronal NOS, such as endothelial or inducible NOS, may be involved in PnPP-19-mediated erectile potentiation. Functional experiments on cavernosal strips using the pharmacological inhibitor ω-conotoxin GVIA (1uM) suggested that the relaxation promoted by PnPP-19 does not depend on N-type $Ca^{2+}$ channels in nitrergic nerve endings, as previously observed with the native toxin PnTx2-6. The involvement of other subtypes of calcium channels and intracellular calcium must be checked.

Given the potential use of PnPP-19 as a drug to treat erectile dysfunction and the lack of information concerning its effect in the nociceptive pathway, the effects of PnPP-19 on nociception was determined. Recent studies of our group showed that this peptide exhibited anti-nociceptive activity, mediated by activation of both opioid and cannabinoid receptors in the peripheral nervous system and this effect appears to involve the inhibition of a neutral endopeptidase (NEP) [40]. Histopathological assays with PnPP-19 showed no signs of toxicity like vascular congestion, edema, cellular necrosis, cytoplasmic vacuolation or nuclear condensation in kidney, heart, liver, lung and brain [37]. PnPP-19 also demonstrated low immunogenicity, even at extremely high concentrations (5 mg/kg) [37]. Table 1 presents a comparison between the effects of the native toxin PnTx2-6 and PnPP-19, on erectile function, in rats.

| | PnTx2-6 | PnPP-19 |
|---|---|---|
| Erectile function effect | improves | improves |
| Dose | 10 nM | 10 nM |
| Effects on sodium channels | Yes | No |
| Action on isolated rat heart | Yes | No |
| Side effects | Yes | Not observed |
| Immunogenicity | Not tested | low |

**Table 1:** A brief comparison between the native toxin PnTx2-6 and the synthetic peptide PnPP-19.

Studies are in progress to check the efficacy of PnPP-19 on rodent animal models for ED. Preliminary experiments (unpublished data) suggest that this peptide is able to improve the impaired erectile function in spontaneously hypertensive rats (SRH). These data encourages and reveals the potential use of PnPP-19 as a pro-erectile drug model, showing promising features for erectile dysfunction treatment.

The treatment of erectile dysfunction with topical formulations has been envisaged as a tendency for the next decades, enabling a comfortable application, with possibly less systemic side effects. Some authors have reported topical formulations for the treatment of erectile dysfunction [41,42]. Recent results of our group have shown that PnPP-19 is able to permeate human skin, enabling the development of a formulation for its topical application. Nano-formulations have been tested, aiming at protecting the peptide from possible degradation and favoring its topical administration (unpublished results).

Nowadays, the treatment of ED is expected to involve drugs that show predictable time of onset and efficacy, are well accepted and easily administered, with few side effects. Studies are in progress to verify the stability of the peptide and it seems to be stable *in vivo*, with a long half-life, since its potentiating effect was observed 15 minutes after intra-peritoneal injection in rats and lasted approximately 1 hour [37]. Besides, PnPP-19 shows no apparent toxicity and low immunogenicity. Furthermore, the synthesis of the peptide is easy and presents a good cost-value, what makes it an excellent and promising candidate for the treatment of erectile dysfunction.

## Conclusions

PnPP-19, a synthetic peptide designed from the tertiary structure of a *Phoneutria nigriventer* toxin (PnTx2-6), is able to stimulate erection through NO/cGMP pathway. As it shows a new mode of action, it can be used as an alternative drug to treat ED, especially for those patients that do not respond to the first-line treatment. This peptide is stable and easily synthesized, shows no apparent toxicity and low immunogenicity and is able to permeate skin, being a good candidate for the development of topical formulations in the treatment of ED.

## Conflict of Interest

The authors declare no conflicts of interest.

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