Some arachnidan peptides with potential medical application

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ABSTRACT: The search for new active drugs that can alleviate or cure different diseases is a constant challenge to researchers in the biological area and to the pharmaceutical industry. Historically, research has focused on the study of substances from plants. More recently, however, animal venoms have been attracting attention and studies have been successful in addressing treatment of accidents. Furthermore, venoms and their toxins have been considered good tools for prospecting for new active drugs or models for new therapeutic drugs. In this review, we discuss some possibilities of using different toxins, especially those from arachnid venoms, which have shown some potential application in diseases involving pain, hypertension, epilepsy and erectile dysfunction. A new generation of drugs is likely to emerge from peptides, including those found in animal venoms.

KEY WORDS: arachnidan toxins, pain, epilepsy, cardiovascular effect, priapism, erectile function, scorpion, spider.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

The search for new drugs presents such great importance in medicine that pharmaceutical industries have invested significant resources in this field. Natural extracts, including those from some plants and animal products, have represented a source for medicines since ancient times. Such extracts usually include a mixture of compounds that can act by affecting different physiological functions, sometimes causing undesirable effects. Besides, some components can constitute potential pharmaceutical models. Nowadays, the focus is on the search for active substances present in natural products, to the detriment of extracts, with to the objective of separating undesirable from desirable components. Obtaining active compounds is now considered far better than developing new extraction methodologies in order to purify and test their activities, most notable among these technologies are mass spectrometry (MS), high performance liquid chromatography (HPLC) and two-dimensional electrophoresis. Similarly, a range of diverse biological assays are available.

Morphine (after Morpheus, the Greek God of dreams) is a classic example of natural compounds useful in medicine. Opium, obtained from poppy juice (*Papaver somniferum*), contains more than twenty distinct alkaloids, including morphine. A reference of its use can be found in the writings of Theophrastus (371-287 B.C.). Opium was introduced into Asia by Arabian traders, mainly to control dysentery. Paracelsus (1493-1541) seems to have popularized the use of opium in Europe. Then, by the middle of the sixteenth century, several uses of opium were appreciated. In 1806, Friedrich Sertürner isolated morphine, a pure substance, which was followed by the isolation of other active alkaloids. These purified substances are nowadays widely used in medicine – for a review, see Reisine and Pasternak (1).

In this context, traditional beliefs should also be considered: for example, alcoholic infusions containing venomous animal parts used to be employed to "treat" human victims of accidents. Additionally, some people believed that eating the head of the venomous animal is an effective treatment against scorpion stings (2)

More recently, some peptides derived from venoms of animals – including scorpions, spiders, amphibians, snakes and marine organisms – have proven to induce analgesia. Many of these peptides selectively inhibit voltage-activated Ca^{2+} and Na^{+} channels, acid-sensitive ion channels (ASIC) or glutamate ionotropic receptors.

The first peptide approved for analgesic therapy (Prialt®, Elan Pharmaceuticals Inc., USA) is a synthetic version of ω-conotoxin MVIIA, a peptide purified from the venom of the fish-eating marine snail *Conus magnus,* which can selectively block N-type calcium channels in pain pathways (3, 4).

Some studies on peptides had also prefigured the design of novel drugs for treating neurological disorders. In this context, epilepsy has been considered a potential focus of attention because it involves neuronal substrates many of which are neurotoxin action sites. Thus, venoms may be particularly useful for neuroprotection and antiepileptic drug research.

A classic example of a successful association between medicine and toxinology is the development of captopril – an antihypertensive drug globally used nowadays that was developed from a toxin model of *Bothrops jararaca* venom (5, 6). Other peptides from different species, such as scorpions, have been studied as possible alternatives models to new antihypertensives.

This review focuses on arachnidan peptides that show a possible application as pharmaceuticals. This paper is solely concerned with describing peptides that present cardiovascular, antiepileptic and antinociceptive effects and also potentiate erectile response.

PEPTIDES WITH ANALGESIC PROPERTIES

Among natural molecules, several peptides from different animals, including arthropods, have recently been studied as antinociceptive agents.

Pain is a complex and multidimensional phenomenon that is subjective and unique to each individual. It is recognized as a multifactorial sensory experience in which several mechanisms are involved. Moreover, its intensity and duration may differ. Unrelieved pain can deeply affect patients' lives and its subjective nature makes it difficult to assess (7).

Nowadays, some neurochemical mechanisms at the spinal cord level are understood, as are the intricate interaction between inhibitory and excitatory neural pathways. There are several potential targets for analgesics including receptors, enzymes, ion channels and signaling molecules. The discovery of subtype-specific toxins that affect different ion/receptor channels has provided targets for pharmacological intervention. A number of novel approaches using animal toxins to relieve pain are currently under investigation. Huwentoxin-I, a N-type calcium

channel blocker isolated from the venom of the Chinese tarantula *Ornithoctonus huwena*, has reduced nociceptive response in a dose-dependent manner when administrated intrathecally at lower doses and its antinociceptive effect was identical to that of ω-conotoxin MVIIA, in the rat model of the formalin test (8).

Another calcium toxin, ω-agatoxin IVA, from the venom of the funnel web spider *Agelenopsis aperta*, has shown selectivity toward P-type calcium channel and enhanced the agonist-induced tail flick antinociception when co-administered spinally with morphine and clonidine (9-11). Phα1β, from the Brazilian armed spider *Phoneutria nigriventer*, has blocked calcium channels when administered spinally in rodent models with acute and persistent pain. Native and recombinant Phα1β has demonstrated an efficient antinociception effect in a thermal model of pain and was more effective than ω-conotoxin MVIIA in preventing and reversing persistent chemical and neuropathic pain (12).

The function of AMPA/kainate receptors (calcium-permeable) on central the nociceptive process has been studied using Joro spider toxin (JSTX) and philanthotoxin, which can reduce allodynia and hyperalgesia when administered intrathecally, indicating a possible involvement of these channels in spinal pain pathways (13-15).

Many other ion channels and receptors are known to mediate some types of pain. For example, voltage-gated sodium channels (VGSCs) play a critical role in modulating the excitability of most neurons, including nociceptive sensory neurons. VGSCs are the target of numerous common analgesics. Recently, new data have proven that loss-of-function mutations in the sodium channel subtype Nav1.7 are the cause of channelopathy-associated insensitivity to pain while gain-of-function mutations are the origin of inherited erythromelalgia and paroxysmal extreme pain disorder (16-22).

Some spider toxins present high selectivity to VGSC subtypes, including ProTx-II, a toxin isolated from the tarantula *Thrixopelma prurient*, which is 100-fold more selective for NaV1.7 than other sodium channel subtypes (23-25). Similar to ProTx-II, some β-scorpion toxins act by reducing channel conductance and interacting with the voltage sensor, though these scorpion toxins may have low affinity for NaV1.7 channels (26). Given the crucial role of this VGSC subtype in human pain perception, ProTx-II may be an attractive tool in the search for novel analgesics.

Several Asian scorpions are commonly employed in Chinese medicine to treat chronic pain, including *Buthus martens* Karsch (BmK). Two venom toxins of this species – namely BmK IT-AP and BmK dIT-AP3 – exhibited analgesic effects on mice. Moreover, BmK dIT-AP3 showed peripheral antihyperalgesia and antinociception in carrageenan induced inflammation in rats (27-29). BmK AS, another toxin from the same venom, also induces peripheral antihyperalgesia and antinociception in carrageenan-induced inflamed rats, possibly by modulating the sodium channel in nociceptors (30). When intrathecally injected, BmK AS markedly reduced formalin-evoked biphasic nociceptive responses in a dose-dependent manner (31). BmK AS1, another similar toxin, produced an antinociceptive effect on the rat peripheral nervous system and spinal cord, this effect was attributed to the modulation of tetrodotoxin-resistant and tetrodotoxin-sensitive sodium channels in peripheral and central neurons (32, 33).

A new class of analgesics found in the venom of the tarantula *Psalmopoeus cambridgei,* psalmotoxin 1, specifically blocks the acid-sensitive ion channel, a proton-gated sodium channel that plays a central role in pathological conditions (34). This toxin presented analgesic properties against thermal, mechanical, chemical, inflammatory and neuropathic pain in rodents. Its action is exerted by blocking ASIC1a and activating the endogenous enkephalin pathway (35).

Channels involved in mechanical hyperalgesia are not fully understood. It was hypothesized that molecules that could block mechanosensitive and stretch-activated channels may reduce mechanical hyperalgesia. Park *et al.* (36) found that GsMTX4, a tarantula toxin from the *Grammostola spatulata* venom, attenuated pain-related response using the Randal Sellito model, but failed to relieve mechanical allodynia evoked by Von Frey hairs. Since mechanical pain is an important clinical problem, GsMTx4 might be useful for treating it. The mechanism underlying the analgesic action of GsMTx4 remains unknown.

Other channels are also involved in different types of pain, such as those from the transient receptor potential (TRP) channels group, e.g. TRPV1, thought to mediate inflammatory thermal hyperalgesia (37), TRPM8 and TRPA1, both described to mediate pain associated with cold (38-40). Thus, molecules that can modulate these targets are of interest – for a review, see Cortright and Szallasi (41).

Diverse pain models and unique times of activation/inactivation of subtype ion-channels may be measured to evaluate the use of these toxins in treating pain. The

development of tolerance to some traditional drugs, such as opioids, can require the use of a combined therapy. Using subtype-specific toxins and other channel modulators that participate in pain might produce an effective analgesia.

PEPTIDES WITH ANTIEPILEPTIC EFFECTS

Epilepsy is one of the most common neurological disorders, and affects 40 to 50 million people throughout the globe (42). It is characterized by the occurrence, over a period of 24 hours, of at least two episodes of excessive and uncontrolled activity of the central nervous system caused by an imbalance between excitatory and inhibitory neurotransmitters (43).

The affected person is predisposed to suffer a convulsion when the basal excitability level of the nervous system increases above a critical threshold (43). Convulsive episodes occur as a consequence of excessive hypersynchronous discharges from neuronal aggregates in the brain (44). A focal seizure is triggered by an abnormal electrical discharge in the cerebral cortex, restricted to a particular area. The clinical expression of this abnormal electrical firing is specific to the stimulated cortical area. The term "seizure" refers to a crisis with predominant motor manifestations (45). Frequently, seizures cause injuries that induce neuronal cell death. There are numerous factors that contribute to this process, such as genetic components and neuronal excitotoxicity, due to excessive release of glutamate, an excitatory neurotransmitter of the nervous system. During a crisis, there is a reduction of the inhibitory neurotransmitter GABA and an increased release of glutamate from presynaptic terminals. Glutamate crosses the synaptic cleft to bind to the N-methyl-D-aspartate (NMDA) glutamate receptors, causing prolonged depolarization and excessive accumulation of intracellular calcium, triggering signaling cascades, including generation of reactive oxygen species (ROS), mitochondrial damage and acute neuronal cell death (46-49).

Nowadays, new anticonvulsant drugs available on the market, act on several molecular targets, including AMPA and NMDA glutamate receptors. Nevertheless, drugs employed for early treatment do not appear to affect the long-term prognosis, especially in relation to epilepsy severity or prolonged remission (50). Moreover, antiepileptic drugs may cause side effects, e.g. chronic toxicity, cognitive impairment, sedation and teratogenesis in chronic patients (51).

Thus, neuroprotective drug leads are extensively researched by the pharmaceutical industry, by seeking new molecules in animal venoms, e.g. cone snails, spiders and scorpions. Many molecules have been isolated from these venoms and studies have shown that they have specific targets in the peripheral or central nervous system, by acting on Ca^{+2} and Na^+ channels and in the glutamatergic system. The observation that these compounds bound to specific targets on neurons constituted evidence that they could possibly lead to antiepileptic drugs (52).

Several toxins isolated from *Phoneutria nigriventer* spider venom bind to ion channels – including PnTx3-3 and PnTx3-4 – and inhibit calcium influx and glutamate exocytosis in isolated nerve endings, thus showing a neuroprotective effect that prevents both neuronal death and loss of neurotransmission in hippocampus CA1 after ischemia induced *in vitro* (53-60). There is evidence that PnTx3-3 and PnTx3-4 also offer neuroprotection *in vivo*, which suggests that these molecules may be used for studies on neurodegenerative diseases, such as epilepsy (61). The Na^+ channel is involved in membrane excitability and is related to several pathologies including epilepsy. Therefore, toxins that bind to this channel may be of interest. There are *P. nigriventer* toxins that act on neuronal Na^+ channel, e.g. PnTx1 and toxins from the PnTx2 group (PnTx2-1, PnTx2-5 and PnTx2-6) (62, 63).

On the other hand, homologous toxins from the PnTx4 group [PnTx4-3, PnTx4(6-1) and PnTx4(5-5)] from *P. nigriventer* are known to interact with the glutamatergic system (64-67). PnTx4(5-5) reversibly inhibits ionic currents mediated through NMDA receptors in rat hippocampal neurons and combined with toxins from the PnTx4 group inhibits glutamate uptake in the micromolar concentration range (66, 67). These toxins appear to be toxic only for insects. Intracerebroventricular injections of high doses (~30 µg) of these toxins in mice did not show any visible effect (64,67). However, pharmacological studies on PnTx4 toxins in mammals are incipient for proposing their possible use as drug models.

Scorpion toxins that act on Na^+ channels can be divided into α-toxins that bind to site-3 and slow or block sodium channel inactivation, and β-toxins that specifically bind to site-4 thus altering the activation of these channels (68, 69). *Buthus martensi* Karsch (*Bm*K) is an Asian scorpion of the Buthidae family, whose venom has been used for treatment of neurological diseases such as epilepsy. Several toxins have been isolated and characterized from this venom, including α and β-neurotoxins, that

present an antiepileptic effect in rats (70). BmK IT2 modulates Na^+ channels in the hippocampus and constitutes an attractive tool for studies on this pathology (71).

The diversity of toxins from scorpions and spiders that act on ion channels, a result of their evolutionary process, represents an arsenal of possible medical drug leads. However, much remains to be done before these molecules can be used in the treatment of neuronal pathologies.

PEPTIDES WITH CARDIOVASCULAR EFFECTS

Cardiovascular diseases are the main cause of death in modern life. Elevated blood pressure reveals an independent, linear and continuous risk of developing cardiovascular diseases (72). Deaths related to coronary heart disease and strokes are mostly caused by hypertension (73).

Several toxins, isolated from different venom sources, act on all cardiovascular system levels, e.g. integrins and desintegrins that disrupt blood coagulation cascade; a toxin from *Androctonus australis garzonii* venom that is able to induce atrial natriuretic peptide secretion; and BmK I toxin, from *Buthus martensi* scorpion venom, that is able to modulate cardiac contraction (74-80). Nevertheless, we will focus on bradykinin-potentiating peptides (BPPs) in this section.

By the end of the 1940s, venom research led to the discovery of bradykinin (BK) by Rocha e Silva *et al.* (81). The authors were studying *Bothrops jararaca* venom and reported that extracts of this snake venom or trypsin, when incubated with the globulin fraction of dog plasma, released a substance that induced contraction of isolated guinea pig ileum. They called this substance bradykinin (from Greek *brady*, slow and *kinesia*, movement) – for a review, see Bhoola *et al.* (82).

The kallikrein-kinin system is involved in the regulation of smooth-muscle contraction, endothelium-dependent vasodilatation, nociception and inflammation. Kinins are produced and accumulated in inflamed tissues, thus triggering the inflammatory process through pain, redness, heat and swelling. These active peptides are formed from their precursors, the kininogens, through the action of kallikreins, a specific serine protease (82).

The broad spectrum of kinin action is mediated by G protein-coupled receptors, pharmacologically classified as B_1 and B_2 subtypes, which present seven transmembrane domains. BK is able to induce vasodilatation, thus decreasing blood

pressure mainly by activating B_2 receptors and triggering the production of nitric oxide (NO), a potent vasodilator (83-87).

Bradykinin-potentiating peptides (BPPs), first described by Ferreira *et al.* (5), present low molecular weight with no disulfide bridges that are able to potentiate the pharmacological effects of BK, since they can induce hypotension and contraction of smooth muscle. Several BPPs are able to inhibit angiotensin-converting enzyme (ACE), a key enzyme in blood pressure control. Therefore, BPPs present great biotechnological interest as potential drugs to treat several cardiovascular diseases. As a matter of fact, the molecular features of BPPs led to the development of Captopril®, the first commercial ACE inhibitor, currently used for cardiovascular diseases treatment including hypertension (6, 87, 88).

After Ferreira's discovery, several BPPs have been isolated from snake venoms. These peptides have a conserved consensus at both extremities, e.g. a pyroglutamic acid residue at the N-terminal and two prolines at the C-terminal (5, 89, 90).

So far, only seven BPPs have been identified in the arthropod venoms (Table 1). In 1993, Ferreira *et al.* (91) isolated and characterized a peptide T from the venom of *Tityus serrulatus*, a South American scorpion. That was the first report of a BPP obtained from arthropod venom. The primary structure of peptide T is quite different from those of other BPPs (Table 1).

Recently, our group has identified a new BPP family, called hypotensins (Table 1). Like all known BPPs, these peptides present no cysteine residues, so that they lack disulfide bridges and comprise unstructured random-coiled peptides, as observed from circular dichroism and 2D-RMN data. The presence of two proline residues at the C-terminal portion was noteworthy, as shown in classic BPPs (92). Besides potentiating BK, TsHpt-I – a member of this family – can induce a transient hypotensive effect, independently of BK administration, and an NO-dependent vasodilatation in aortic ring preparations (92). Diverging from all known BPPs, TsHpt-I acts as an agonist in relation to kinin receptors and does not inhibit ACE. A structural-functional study was performed and demonstrated that the C-terminal doublet, Pro-Pro, of these molecules was important to potentiate BK as well as the positively charged lysine residue, just before Pro-Pro residues, was crucial for activation of kinin receptors by these peptides.

Table 1 – BPPs isolated from arthropod venoms

Names	Species	Primary structures	References
BPP-S	*Scaptocosa raptoria*	<EAPWPDTISPP_	93
K$_{12}$	*Buthus occitanus*	LRDYANRVINGGPVEAAGPPA	94
Peptide T	*Tityus serrulatus*	KKDGYPVEYDRAY	91
TsHpt-I	*Tityus serrulatus*	AEIDFSGIPEDIIKQIKETNAKPPA	92
TsHpt-II	*Tityus serrulatus*	AEIDFSGIPEDIIKEIKETNAKPPA	92
TsHpt-III	*Tityus serrulatus*	AEIDFSGIPEDIIKQIKETNAKPP_	92
TsHpt-IV	*Tityus serrulatus*	AEIDFSGIPEDIIKEIKETNAKPP_	92

<E: pyroglutamic acid; alignments were obtained from PP residues.

PEPTIDES THAT POTENTIATE ERECTILE FUNCTION

Erectile dysfunction (ED) is defined as the persistent inability to achieve or maintain an erection sufficient for a satisfactory sexual performance (95). Many factors can contribute to the development of ED including smoking and vascular diseases such as atherosclerosis, hypertension, *diabetes mellitus*, besides anxiety and depression (96-102). The use of specific pharmacological medications, including antihypertensive drugs, diuretics, cardiac medications, hormones and antidepressants can induce ED. Nowadays, a profound change has occurred in current strategies for the pharmacological treatment of ED, mainly with the advent of effective oral erectogenic drugs (103).

Penile erection is a neurovascular phenomenon that depends upon neural integrity, functional vascular system and healthy cavernosal tissue (101, 103, 104). The penis is innervated by both autonomic and somatic nerve fibers. Sympathetic and parasympathetic nerves from the pelvis ramify to form the cavernous nerves (101, 104). Upon sexual stimulation, neurotransmitters are released from cavernous nerve terminals and smooth muscle endothelium. Similarly, vasoactive relaxing factors from the endothelial cells of the penis, which relax arteries and arterioles supplying the erectile tissue, increase the penile blood flow and ultimately produce a penile erection (101, 103, 105).

Cholinergic nerves, noradrenergic nerves, noncholinergic and nonadrenergic nerves (NANC), nitric oxide (NO), and other factors such as vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP) mediate smooth muscle relaxation

of the corpus cavernosum (106, 107). Moreover, sheer stress and muscarinic acetylcholine receptors on the trabecular endothelium stimulate the production of NO, an important factor for penile erection (98).

The nitric oxide pathway is of critical importance in physiological induction and maintenance of erection (107-109). NO is synthesized from L-arginine by the action of NO synthase (NOS) – three isoforms of this enzyme are known: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS) (109, 110). After being synthesized, NO is released and diffuses into smooth muscle cells, activating the soluble form of guanylate cyclase enzyme, thus increasing the concentration of cyclic guanosine monophosphate (cGMP). The latter activates cGMP-dependent ion channels, reducing cytosolic calcium via sequestration, extrusion and opening of potassium channels, which result in hyperpolarization of smooth muscle cells of the corpus cavernosum, in addition to activating myosin chain phosphatases. Finally, increased cGMP leads to smooth muscle relaxation, increased arterial flow, and corporal venous-occlusion with a subsequent penile erection. The hydrolysis of cGMP by phosphodiesterase type 5 (PDE 5) leads to a corpus cavernosum contraction (103, 108, 111-116).

A corpus cavernosum contraction results in penile flaccidity and is maintained by increased intracellular calcium, which binds to calmodulin leading to change in its conformation, exposing sites of interaction with myosin-light-chain kinase (MLCK). Kinase catalyses phosphorylation of myosin light chains that activate myosin ATPase, hydrolyzing ATP in such a way as to provide energy for muscle contraction. In another pathway, after cytosolic calcium returns to basal levels, the activation of excitatory receptors coupled to G protein (Rho A) can also cause contraction. Additionally, Rho A activates Rho-kinase, which phosphorylates and thereby inhibits the regulatory subunit of smooth muscle myosin phosphatase and prevents dephosphorylation of myofilaments, thus maintaining a contractile tone (116, 117).

Nowadays, the main pharmacotherapy for treating ED uses PDE5 inhibitors, including sildenafil (Viagra®), taladafil (Cialis®) and vardenafil (Levitra®) (99, 101, 113, 116). The action mechanism of these inhibitors requires intact NO-relaxing nerve fibers and healthy corpus cavernosum endothelium. The drugs inhibit the phosphodiesterase type5, thus preventing hydrolysis of cGMP and consequently maintaining erection. Moreover, PDE5 inhibitors, mainly sildenafil, are not efficient in

treating patients with radical prostatectomy or vascular diseases, such as diabetes, where NO production is impaired (113).

Interestingly, peptides present in animal venoms, as scorpions and spiders, among other symptoms, cause priapism – a painful penile erection that is persistent or unrelated to sexual stimulation (118). These venoms, as well as some peptides purified from them, have shown efficiency in promoting priapism and erection in different experimental models (53, 115, 116, 119-121).

Animal venoms or purified toxins evoke complex effects on ion channels, mainly sodium, potassium and calcium channels (56, 66, 122-127). Experimental approaches using bioassay cascade have demonstrated that crude venom from the scorpion *Tityus serrulatus* causes relaxation in rabbit and human cavernosal smooth muscle *in vitro*, by a mechanism dependent on NO release from nitrergic nerves, and this effect was decreased with non-selective NOS inhibitor, N(omega)-nitro-L-arginine methyl ester (L-NAME) (127, 128). Additionally, Ts3, a toxin isolated from this venom, causes relaxation of NO-dependent corpus cavernosum in rabbits, a response blocked by L-NAME and tetrodotoxin (TTX) (129). Other scorpion venoms, as those from *Androctonus australis* and *Buthotus judaicus*, have also been reported to cause relaxation of rabbit corpus cavernosum. This effect was inhibited with L-NAME, 7-nitroindazole, 1H-[1,2,4] oxadiazole [4,3-alquinoxalin-1-one] and TTX (119). Spider venoms are composed of distinct proteins, peptides and biologically active molecules, most of them neurotoxins (56). These venoms have been described as potentially useful for future discovery and development of new biologically active molecules with medical application (66, 115, 116, 122). The crude venom of *Phoneutria nigriventer* contains potent neurotoxins that cause excitatory symptoms (53, 56). Some of these toxins, namely PnTx2-5 and PnTx2-6, share 89% of similarity in their amino acid sequence and have been observed to stimulate relaxation of the corpus cavernosum smooth muscle in rabbits, rats and mice, inducing penile erection (54, 115, 116, 121, 130, 131). Both toxins were described as site-3 toxins, according to their effects on sodium channels (125, 132). As observed for scorpion toxins, priapism induced by these toxins has been directly associated with the nitric oxide pathway in rats and mice and this effect has been inhibited by L-NAME (115, 116, 131).

Nunes *et al.* (115) proposed that improved effect of PnTx2-6 on rat penile erection seems to be mediated by relaxation of vasculature and smooth muscle in the corpus

cavernosum via NO release from NANC nerve terminals. Moreover, there is evidence that specific receptors for the toxin exist in the corpus cavernosum, following this tissue labeling after injection of PnTx2-6 labeled with technetium-99 (115).

Another study has demonstrated that some genes are under-expressed and others in the oxide nitric pathway are over-expressed, as ednrb that activates the L-arginine/NO/cGMP pathway and is involved in the relaxation of the corpus cavernosum (133). This study strengthens the hypothesis by Nunes *et al.* (115) that the priapism induced by PnTx2-6 is a consequence of a highly specific interference of this neurotoxin with the NO pathway. The difficulty in obtaining these toxins is a limiting factor for their use. Constructing recombinant toxins, using protein engineering techniques, is an important alternative to obtain sufficient material and can overcome these problems.

The cDNA for PnTx2-6 toxin from *Phoneutria nigriventer* venom was cloned and expressed as a thioredoxin fusion protein in the cytoplasm of *Escherichia coli* and the erectile response, as with native PnTx2-6, was significantly potentiated after subcutaneous injection with the recombinant PnTx2-6. There is clear evidence of the pharmacological and therapeutic potential uses of these toxins from animal venoms for ED treatment. However, many studies are still necessary, due to the high toxicity of these molecules and the different collateral effects that they may provoke. In addition, we believe that these molecules might constitute adequate models for the design of other therapeutic drugs, targeting different sites not yet explored for potentiating erectile function or treating erectile dysfunction.

FINAL REMARKS

The aforementioned peptides that exhibit analgesic, antiepileptic and antihypertensive activities and potentiate erectile dysfunction represent only a small sample of a plethora of molecules from arthropods that have been explored due to their potential application in medicine and in biotechnology (116, 134-137). Furthermore, arthropods have presented molecules with potent antibiotic and insecticide effects, as well as properties for treating autoimmune diseases and some myotonic dystrophy (123, 135, 138-141).

Venoms, including those from arthropods, showing biological activities with various potential uses in medicine and biothecnology have recently been reviewed (142).

It is hoped that a new generation of drugs based on active peptides can be produced in the next future. Arachnidan and other animal venoms revealed a new source for the discovery and formulation of active drugs.

Finally, venoms, for a long time considered dangerous and exotic substances, now prove to be potentially new sources to cure different diseases.

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