We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,400
Open access books available

117,000
International authors and editors

130M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

New Perspectives in Drug Discovery Using Neuroactive Molecules From the Venom of Arthropods

Márcia Renata Mortari and
Alexandra Olimpio Siqueira Cunha

Additional information is available at the end of the chapter

1. Introduction

Arthropods are one of the most ancient groups of animals in earth and their venoms have been responsible for their chemical defense in a very efficient way. Resulting from an intense and elaborated evolutionary process, venoms produced by arthropods have a very complex repertoire of biologically active molecules. When inoculated in mammals these molecules induce a wide range of systemic effects, including actions in the CNS. In mammalian CNS, venom compounds may either inhibit or stimulate with affinity and specificity structures such as: ion channels, neurotransmitter receptors and transporters [1-3]. Not surprisingly, these actions have attracted the attention of many investigators in search of tools to help the understanding of neural mechanisms as well as those in search of novel probes in CNS drug design for the last 20 years [3,4]. In addition to the growing interest in finding new neuroactive compounds, the improvement of proteomic and transcriptome techniques has stimulated great progress in the bioprospecting, enabling and accelerating the testing of new toxins in several animal models. Animal research aiming at the efficacy of peptides and acylpoliamines, isolated from arthropod venoms, have revealed the great potential of these compounds to treat various diseases, such as epilepsy, Parkinson's, Alzheimer's, chronic pain and anxiety disorders

According to World Health Organization (WHO), neurological and mental disorders are one of the greatest threats to public health not only for its direct and immediate effects, but also for the progressive nature of these diseases, often leading to disability and death [5]. The symptoms of most of these diseases are often well treated with a several pharmaceuticals, such as antidepressants, anxiolytics, anticonvulsants and analgesics. However, it is well known that neuroactive drugs may induce a complex range of adverse effects that limit the

usage in some patients or may even function as a factor of impairment in people's quality of life. According to [6], none of antiepileptic drugs discovered in the last 20 years, was efficient to cure or even suppress seizures in epileptic patients. Therefore, there is a continued need for the discovery of novel drugs to treat most neurological and mental disorders [7].

This chapter will target the discussion of recent contributions of research on the compounds of arthropod venom, for the discovery of novel tools to study the functioning of the structures of mammalian CNS, as well as the supply of novel alternatives to the treatment of neurological disorders. Among the major compounds, it will be highlighted those with the analgesic, anxiolytic, antiepileptic and neuroprotective effects, with emphasis on the most promising on preclinical or clinic evaluation.

2. Main targets of the neuroactive compounds isolated from arthropod venoms

Venom isolated from bee, scorpion and spider have been used to the treatment of various diseases in Chinese and Korean traditional medicine, such as epilepsy, stroke, facial paralysis, arthritis, rheumatism, back pain, cancerous, tumors, and skin diseases [8-10]. Moreover, venoms of arthropod animals have been used to study various physiopathological processes, and also offer opportunity to design and develop new therapeutic drugs [3,11,12] .

Arthropod venoms are rich in biologically active substances with different physiological actions, specially the neurotoxins. So far, identified neurotoxins generally comprise the classes of peptides or acylpolyamines, acting with affinity and specificity over excitatory or inhibitory neurotransmissions (for revision see [12]. The actions of these compounds include the interaction with Na^+, K^+ and Ca^{2+} ion channels, agonism or antagonism of metabotropic and ionotropic receptors for neurotransmitters as the excitatory neurotransmitter glutamate. At the presynaptic level, several studies have shown the interaction of arthropod neurotoxins with protein transporters of neurotransmitters, resulting in the facilitation or inhibition of their uptake.

3. Antinociceptive effects

Of extreme importance for the organism, pain is an indicator of corporal integrity and has been considered since January 2000, by the Joint Commission on Accreditation on Healthcare Organizations (JCAHO) as the fifth vital sign that should be assessed and recorded together with other signals immediately after birth. According to the International Association for the Study of Pain (IASP), pain is defined as an unpleasant sensation and emotional experience associated with actual or potential tissue damage. However, approximately one third of world population suffers from pathological persistent or recurrent pain, which is a common complaint in patients with different diseases, and exerts great impact on their social life [13]. In these cases, treatment is a challenge for researchers and health professionals who

constantly seek new therapeutic strategies, since most of these are inadequate or cause serious side effects [14].

Analgesics and systemic conservative therapies are widely used for pain control. However, in many cases, especially in patients with neuropathic pain, more aggressive treatments are needed, which promote a significant clinical improvement but only in 30-50% of patients [15,16].

Although an injection of arthropod venoms is commonly reported to cause tonic pain and hyperalgesia, there is also evidence suggesting that these venoms might have antinociceptive effects on inflammation. Thus, nowadays, toxins isolated from arthropods are considered powerful tools, since they have congruent targets of the impulse transmission of pain, and may provide an attractive alternative to opioid treatments.

3.1. Polypeptide toxins from Scorpion

The most studied Arthropod venom is extracted from the Asian scorpion *Mesobuthus martensi* Karsch (BmK). It is composed of several toxins, and so far, ten have been described, which produce powerful antinociceptive effects. This is the case of the two β-excitatory anti-insect toxins BmK IT-AP (or Bm33-I) and BmK AngP1, two β- depressant anti-insect toxins BmK dITAP3 and BmK IT2, as well as six toxins yet without consensus classification, BmK AS, BmK AS1, BmK AGAP, BmK Ang M1, BmK AGP-SYPU1 and BmK AGP-SYPU2. These compounds probably belong to a family of peptides NaScTx that are composed of 60-76 amino acid residues with four disulfide bonds, the cysteine positions among these toxins are highly conserved [17,18]. Considering their structures, they might be able to bind to sodium channels impairing depolarization of the action potential in nerve and muscle, resulting in neurotoxicity [18], although it remains to be fully investigated.

The NaScTx family can be classified in at least two major families, α and β, according to the mode of action on Na$^+$ channels [19]. The binding of α-toxins delays Na$_v$ channel inactivation, while that of β-toxins shifts the membrane potential dependence of channel activation to more negative potentials. α and β-toxins also exhibit pharmacological preferences for mammals or insects sodium channels. Therefore, considering their pharmacological activities, α and β NAScTx can be also divided into three groups:

i.   "classic" highly specific for mammals;

ii.   "α-like toxins" active both on mammals and insects, which are far less specific and less active than the "classical" ones;

iii.   α-toxins only specific for insects and without any toxicity on mammals, even at high concentrations. Moreover, the insect selective β-toxins have been divided into two groups: the excitatory insect toxins and the depressant insect toxins.

Regarding the β-excitatory anti-insect toxins, BmK IT-AP (Insect Toxin-Analgesic Peptide), which was isolated in 1999, produces a potent antinociceptive effect in mouse-twisting model, after i.v. injection [20]. The same toxin has also been sequenced by another group and named Bm K 33-I [21]. Later, Guan and colleagues [22] identified a novel toxin with analgesic effects, BmK AngP$_1$, which shows an evident analgesic effect with simultaneous excitato-

ry insect toxicity, but is devoid of any toxicity on mice even at high dosages. The analgesic effect was assessed with a mouse-twisting model. The analgesic effect on mice of the AngP$_1$ is at least 4-5 times weaker than that of IT-AP, but the toxicity to insects is twice as strong as that of IT-AP [20,22]

In relation of depressant toxins isolated from BmK venom, BmK IT2 has been more studied from the venom of BmK (Fig 1). Intraplantar injection of BmK IT2 inhibited thermal hyperalgesia in carrageenan-treated rats and significantly prolonged paw withdrawal latency in normal rats [23]. This toxin also displays an inhibitory effect on the C component of the rat nociceptive flexion reflex by subcutaneous injection in vivo [24]. Peripheral or spinal delivery of BmK IT2 suppressed formalin-induced nociceptive behaviors and c-Fos expression in spinal cord [25,26]. Both BmK IT2 and Bm K dIT-AP3 (depressant Insect Toxin-Analgesic Peptide 3) are toxic for insects, but not for mammals [27], and shows 86.7% of sequence similarity [23]. BmK dIT-AP3 also induces analgesia in the mouse-twisting model [18]. Using whole-cell patch clamp, it has been shown that BmK dIT-AP3 inhibits Na$_v$ currents of rat dorsal root ganglion (DRG) neurons, blocking more selectively the tetrodotoxin-resistant (TTX-R) component of the Na$^+$ currents. These results suggest that the inhibition of the rat nociceptive flexion reflex by BmK dITAP3 may be attributed to modulation of the DRG's voltage-gated Na$^+$ channels [24].

Wang and colleagues [28] isolated a new antinociceptive peptide, named BmK AGP-SYPU1. Recombinant BmK AGP-SYPU1 showed similar analgesic effects on mice compared to natural when assayed using a mouse-twisting model [28]. More recently, BmK AGP-SYPU2 was purified and tested, also in mouse-twisting model. Sequence determination showed that the mature BmK AGP-SYPU2 peptide is composed of 66 amino acid residues, and BmK AGP-SYPU2 is identical to BmK alpha2 and BmK alphaTX11.

BmK AS had a strong analgesic effect on both visceral and somatic pain [29,30]. It relieves formalin-induced two-phase spontaneous flinching response and carrageenan-induced mechanical hyperalgesia, probably by modulating the voltage-gated Na$^+$ channels of sensory neurons [31,32]. Moreover, BmK AS showed activity nearly equivalent to that of morphine. Later, a new peptide that possesses 86.3% of similarity with BmK AS was identified. Both polypeptides have 66 amino acids cross-linked by four disulfide bridges [29]. In addition, these two peptides show a poor similarity with other known types of scorpion toxins. BmK AS and AS1 are not toxic against mammals and only have a weak toxicity to insects. BmK AS, then BmK AS1, have been found to significantly stimulate the binding of [3H]-ryanodine to partially purified ryanodine receptors [33]. More recently, electrophysiological studies have shown that they are able to inhibit Na$^+$ currents in NG108-15 cells [34] and to depress TTX-sensitive and TTX-resistant Na$^+$ currents in rat small DRG neurons. Interestingly, in rat models, BmK AS1 also displays antinociceptive effects according to [33]. These authors concluded that the effects could be mediated by the modulation of voltage-gated Na$^+$ channels and they also suggested that BmK AS and BmK AS1 could form a new family of scorpion insect toxins.

BmK AGAP (antitumor-analgesic peptide), isolated in 2003, had strong inhibitory effect on both viscera and soma pain [35]. To evaluate the extent to which residues of the toxin core

contribute to its analgesic activity, nine mutants of BmK AGAP were produced and tested. However, further studies are necessarily to elucidate the mechanism of action as well as to exploit its analgesic activity [36]. In relation to BmK Ang M1 [37], it also was reported to exhibit potential analgesic effect. Moreover, electrophysiological studies showed that BmK AngM1 at the concentration of 1 μM inhibited voltage-dependent Na$^+$ current (I_{Na}) and voltage-dependent delayed rectifier K$^+$ current (I_K), but had no effects on transient K$^+$ current [37].

It is important to note that the excitatory and depressant anti-insect toxins belong to different groups, which have distinct modes of interaction with receptors. Thus, one can infer that the analgesic effect of these peptides may have a molecular mode and mechanism different from that of insect toxicity. Still, the mechanisms by which these scorpion toxins can modulate pain pathways remain to be clarified. According to [8], four different possibilities might be described:

i.   peptides act directly Na$^+$ channels involved in the pathway of pain,

ii.   peptides modulate indirectly the pain sensation,

iii.   peptides also modulate other targets involved in pain pathway

iv.   pain alleviation is only apparent and results from misinterpretations that might have occurred from animal models used.

3.2. Polypeptide toxins from Spider

Another group of arthropods that have very promising antinociceptive compounds are spiders [41]. In 1996, Roerig & Howse reported the effect of ω-agatoxina IVA (Fig 1) isolated from funnel spider *Agelenopsis aperta* venom, against thermal stimulation in the tail flick test, when co-administrated with morphine intrathecal. Intrathecal injection of ω-agatoxin IVA (0.2 nmol/kg) also decreased the licking time in both the early and late response phases in a dose-dependent manner in the Formalin test [42]. The use of this peptide as an analgesic could be of particular benefit in patients tolerant or opioid-dependent, since this compound exhibits selectivity for the P/Q Ca^{2+} channels [43]. Other spider venom very promissory is the venom of the Brazilian armed spider *Phoneutria nigriventer*, the purified fraction 3 (PhTx3) contains 6 toxin isoforms (Tx3-1 to -6) [44,45] that target Ca^{2+} channels with different affinity patterns. Moreover, one toxin, Tx3-6 (Phα1β), demonstrated that it preferentially blocks the N-type calcium current [46] and produce a potent antinociceptive effect with higher therapeutic index [44]. Dalmolin and colleagues [45] showed that Tx3-3 (purified the same fraction) caused a short-lasting antinociceptive effect in the nociceptive pain test and a long-lasting antinociceptive effect in neuropathic pain models, without producing detectable side effects. However, Tx3-3 did not change the inflammatory pain. Tx3-3 blockade of P/Q- and R-type Ca^{2+} channels and inhibit the glutamate release in rat brain cortical synaptosomes [47]. Other neurotoxin isolated from spider *Phoneutria nigriventer* is Phα1β, which is a potent toxin blocking neuronal voltage-sensitive Ca^{2+} channels. This peptide induced longer antiallodynic effect than μ-conotoxin MVIIA and morphine in mice [48].

In addition to toxins calcium modulators, compounds isolated from spider that interact with other ionic channels have shown great potential. A new class of peptide toxins named is the Huwentoxin I (HWTX-I, Fig 1) that is the most abundant toxic component in the crude venom of the Chinese bird spider *Ornithoctonus huwena*. Whole-cell patch clamp records revealed that HWTX-I selectively inhibits N-type Ca^{2+} channels in NG108-15 cells, and it also can block transmitter release from nerve endings by preventing depolarization induced by calcium influx [38] Antinociception effect of the HWTX-I in formalin test was greater and lasted two-fold longer time compared to morphine [39]. Furthermore, Tao and collaborators [40] demonstrated that intrathecal administration of HWTX-I is effective in antinociception in the rat model of rheumatoid arthritis more effective than ibuprofen.

Several studies have reported that intrathecal administration of non-selective blockers of Ca^{2+} channels shows antinociceptive effects in animals tested with thermal stimuli: hot plate and tail flick. According to [49], N and P/Q Ca^{2+} channels are probably involved in nociceptive behavior induced by formalin injection in rats, while the L-type channels has no effect. N- and P/Q-type Ca^{2+} channels are expressed specifically in the nervous system, and they have a major importance in controlling the excitation of spinal neurons from sensory afferents of inflamed tissues, relieving inflammatory pain.

A new class of peptide toxins named π-theraphotoxin-Pc1a (π-TRTX-Pc1a; also known as psalmotoxin-1 (PcTx1) was isolated from the venom of the spider neotropical *Psalmopoeus cambridgei* (Fig.1). π-TRTX-Pc1a is the most potent and selective blocker of ion channels sensitive to acid – ASICa [50]. These channels play important roles in pathological conditions such as cerebral ischemia or epilepsy, as well as being responsible for the sensation of pain that accompanies tissue acidosis and inflammation [51]. Since external acidification is a major factor in pain associated with inflammation (hematosis muscle and cardiac ischemia, or cancer), these neurotoxins can be used to control the pain sensation triggered by these channels [52]. π-TRTX-Pc1a was shown to be an effective analgesic, comparable to morphine, in rat models of acute and neuropathic pain when injected directly in Central Nervous System [53] and intranasal administration of this peptide resulted in neuroprotection of neurons in a mouse model of ischemic stroke even when administered hours after injury [54].

Other important target in the search for new analgesics isolated from spider venoms are Na_V channels, since modulatory compounds of these channels are the dominant pharmacological species in spider venoms, although still poorly characterized. In this context, Intrathecal administration of β-TRTX-Gr1b (formerly GsAFI), a peptide obtained from venom of *Grammostola spatulata*, the Chilean pink tarantula spider, induced analgesia in a variety of rat pain models such as the tail flick latency test, hot plate threshold test, von Frey threshold test, and formalin pain test, without any confounding side-effects. Moreover, the β-TRTX-Gr1b peptide did not exhibit cross tolerance with morphine [55].

Further on spider venoms, Purotoxin-1 (PT1) was recently isolated the, from the venom of the Central Asian spider *Geolycosa sp* [56]. PT1 is a 35-residue peptide with four disulfide bonds, and it exerts a potent analgesic effect in rat models of acute and chronic inflammatory pain by injection of either carrageenan or Freund's complete adjuvant, respectively. PT1 was also effective in reducing the number of nocifensive events triggered by the injection of capsaicin

or formalin (only second phase) [56]. This molecule also inhibits P2X3 receptors in a power‐ful and selective manner. These ATP-activated receptors are largely expressed in mammali‐an sensory neurons play a key role in the pain perception. Thus, PT1 appears to be a promising lead compound for the development of analgesics that target these receptors [56].

3.3. Polypeptide toxins from Bees and Wasps

Bee venom has been traditionally used to relieve pain and treat chronic pain diseases (for revision see [57]). Moreover, acupoint stimulation into the subcutaneous region (acupunc‐ture) rather than other injection sites may be important for the antinociceptive effects of this venom. There is increasing evidence suggesting that bee venom has antinociceptive effects on visceral nociceptive effects, mechanical and thermal hyperplasia, formalin-induced pain behavior and collagen-induced arthritic pain, as well as knee osteoarthritis (OA)-related pain [58-63]. BV contains at least 18 active components, including enzymes, peptides, and biogenic amines, which have a wide variety of pharmaceutical properties, and so multiple mechanisms associated to antinociceptive effects have been suggested, such as activation of the central and spinal opioid receptor and α_2-adrenergic receptor, as well as activation of the descending serotonergic pathways (for revision see [64]).

Melittin is a small protein containing 26 amino acid residues and is the major bioactive com‐ponent in BV (Fig.1). This polypeptide readily integrates into and disrupts both natural and synthetic phospholipid bilayers [65,66]. Melittin also enhances the activity of PLA$_2$ [67] and has a variety of effects on living cells possibly through the disruption of the membrane [68]. The decrease in cyclooxygenase (COX)-2 and phospholipase PLA$_2$ expression and the de‐crease in the levels of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, nitric ox‐ide (NO) and oxygen reactive species (ROS) are suggested to be associated with the anti-arthritis effect of melittin [69]. This peptide has also been thought to play a role in production of anti-nociceptive and anti-inflammatory effects [64]. In addition, Merlo and colleagues [70] demonstrated the antinociceptive activity of the melittin in experimental models of nociceptive and inflammatory pain. Interestingly, melittin failed to increase the latency for the nociceptive response in the hot-plate model and in the first phase of the for‐malin test, revealing that melittin presents an activity that resembles more that of anti-in‐flammatory drugs and less that of centrally acting drugs [70]. Nevertheless, the molecular and cellular mechanisms underlying the anti-nociceptive effects of melittin are not entirely clear and remain to be further clarified by further experimental studies [57].

Addition of melittin, adolapin has been isolated from BV and it demonstrated a potent anal‐gesic effect in mouse-twisting model and the Randall-Sellito's test [71]. The anti-inflammato‐ry activity of adolapin was evaluated and it had a pronounced activity in the following tests: carrageenan, PG, adjuvant rat hind paw edema and adjuvant polyarthritis. The effects of adolapin are presumably due to its ability to inhibit the prostaglandin synthesis via inhibi‐tion of cyclooxygenase activity [71,72].

Venoms of wasps also have analgesic peptides. Mortari and colleagues [73] isolated a com‐pound with antinociceptive activity from the venom of the Brazilian social wasp *Polybia occi‐dentalis*. The isolated peptide is a neurokinin named Thr[6]-Bradykinin. This neurokinin is a

small peptide consisting of nine amino acid residues, Arg-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg-OH, which exhibits a high degree of homology with bradykinin (BK), except for the substitution of Thr for Ser in position 6 at BK. As a result, small changes in their secondary structures are observed [74]. This modification has been regarded as responsible for increasing B_2 receptor affinity and potency of Thr[6]-BK in relation to BK *in vitro* and *in vivo* [74,75]. Thr[6]-BK antinociceptive effect was dose- and time- dependent, when injected directly into the CNS of rats in hot-plate and tail-flick tests, and it was three times more potent than morphine and 4 times more potent than BK in tail-flick test. Thr[6]-BK induced antinociception by activating presynaptic B_2 receptors, which activate descending adrenergic pathways. Studies investigating the role of kinins in the CNS provide new information on the supraspinal system of the pain control, whose modulation may represent a new strategy to control pain-related pathologies [76].
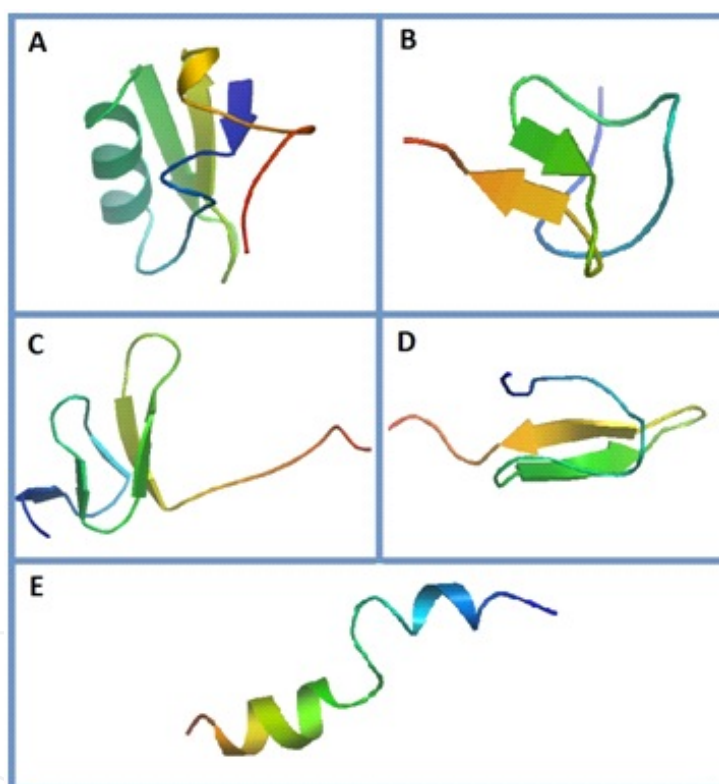

Figure 1. Tridimensional structure of antinociceptive peptides isolated from arthropod venoms. (A) BMK IT2; (B) HWTX 1; (C) ω-Agatoxin IVA; (D) π-Theraphotoxin-Pc1a; (E) Mellitin. Uniprot entry code: P68727, P56676, P30288, P60514 and P01501, respectively.

Besides peptides, some studies have evaluated the analgesic activity of acylpolyamines that can be used as new alternative drugs for the treatment of chronic pain, as well as tools for the study of the functional role of the AMPA/kainate receptors in the processing of nociceptive pain [77]. In this regard, intrathecal administration of different doses of these toxins blocked thermally induced allodynia [78] and hyperalgesia [79]. The effect of these neuro-

toxins may suggest a possible involvement of AMPA receptors in the spinal cord during the nociceptive excitatory stimulation [80,81].

4. Antiepileptic and neuroprotective effects

Neurodegenerative disorders comprise a wide range of conditions mostly characterized by a progressive loss of neuronal function and neuronal cell death. The incidence of these diseases in population differs greatly. In conditions such as Parkinson disease and Alzheimer, the number of cases significantly increases in elderly, whereas epileptic patients are mostly children and adolescents. Many processes may trigger neuronal cell death, such as trauma, stroke, tumors, infections, genetic factors and biochemical alterations. Among the latest, the alterations in Ca^{2+}-mediated signaling is thought to play a key role in many neurodegenerative disorders and the increase in intracellular Ca^{2+} concentration might alter neuronal membrane potential [82]. Moreover, the hyperactivation of excitatory transmission mediated mostly by L-glutamate and its ionotropic receptors; kainate, AMPA and NMDA, is responsible for the excessive cationic influx that depolarizes neuronal cells and lead to sustained hyperexcitation observed in brain pathologies such as epilepsy [83]. This increase in glutamatergic activity often referred to as glutamate excitotoxicity [84], might also involve non-receptor neurochemical events such as failure in glutamate uptake system, which ends with an increase in the availability of this neurotransmitter in the synaptic cleft [85,86]. The importance of L-glutamate in neurological disorders relies on the fact that this neurotransmitter is release in the great majority of fast synapses in CNS [84,83]. In this context, many molecules mostly peptides and acylpolyamines, acting on ion channels, receptors and transporters were isolated from arthropod venoms, remarkably spiders, scorpions and wasps [3]. According to [82], polyamines are non-specific antagonist of ligand-gated ion channels, acting at glutamatergic and Ach receptors in an uncompetitive way, that is, the receptor must be activated in order to occur the blockade. This mode of action might diminish the side effects of newly designed medicines, since it blocks only the activated receptors, but does not prevent their opening.

The venom of the orb-web spider *Nephilia clavata* was one of the first venoms studied during the 80s, which resulted in the identification of small compounds named acylpolyamines, among whose we may find jorotoxin (JSTX), one of the first glutamate receptor uncompetitive antagonists [83,84]. Together with JSTX, another polyamines such as argiopin from the venom of the spider *Argiope lobata* [85] and philantotoxin (PhTx) from the venom of the solitary wasp *Philanthus triangulum* [86]. Following the structural characterization and studies in insect or crustaceans, the reports on the action of these polyamines in mammalian CNS started to take place, mostly during the 90s [87]. JSTX-1 and JSTX-3 are synthetic analogues of JSTX. The first inhibits kainate-induced seizures, whereas the latter block glutamate release and hippocampal epileptic discharges [88,89]. Later, JSTX-3 was shown to inhibit the formation of superoxide dismutase-1 (SOD-1) aggregates that lead mutant motor neurons (mSOD-1) to death during the familiar form of the neurodegenerative disease, amyotrophic lateral sclerosis [90]. The authors concluded that increased Ca^{2+} influx mainly through AM-

PA/kainate glutamate receptors make mutant neurons more vulnerable to damage and therefore, JSTX-3 is an interesting neuroprotective agent in this model.

The fraction of the venom of the spider *Agelenopsis aperta* containing argiotoxin, was first demonstrated to have anticonvulsant in NMDA-induced and audiogenic seizures [91]. The synthetic analogue of argiotoxin, Arg-636, is a selective antagonist of NMDA receptors binding to the Mg^{2+} binding site at the receptor with anticonvulsant and neuroprotective actions. In addition, from the venom of *A. aperta*, another NMDA receptor blocker, Agatoxin 489 was reported as anticonvulsant against kainate-induced seizures and its synthetic analogue Agel-505, was able to block cationic currents in oocytes transfected with NMDA receptor cDNA [92].

Aside from antagonizing glutamate receptors, arthropod neurotoxins may exert anticonvulsant and neuroprotective effects targeting other neurotransmitter systems. The venom of the Brazilian spider *Phoneutria nigriventer* has been extensively studied over the past 20 years. Neurotoxins isolated from the venom of *P. nigriventer*, such as PhTx-3 (Tx-3) were reported to inhibit Ca^{2+} dependent-glutamate release [47]. Tx3-3 and Tx3-4 also inhibit voltage-activated Ca^{2+} channels of P/Q type [93] and recently their neuroprotective activity was tested. According to [94], Tx3-3 and Tx3-4 protected hippocampal slices against damage and cell death induced by ischemic insult resulted from low oxygen and low glucose. Moreover, PhTx3, Tx3-3, and Tx3-4, inhibited cell loss in retinal slices submitted to the same ischemic protocol [95]. Another Brazilian species lives in Cerrado, the colonial spider *Parawixia bistriata* and has many neuroactive molecules with different modes of action [96]. Parawixin-1 was the first isolated neurotoxin from *P. bistriata* venom. In experiments using rat retinas, submitted to ischemic insult, the intravitreal injection of Parawixin inhibited cell loss [97], probably through a potent and specific enhancing action on glutamate transporters type EAAT2 [98]. Another neurotoxin isolated from the venom of *P. bistriata*, Parawixin II, formerly, FrPbAII, inhibited GABA and glycine uptakes in synaptosomes from rat cerebral cortices. In addition, the administration of Parawixin II into the vitreous humor of Wistar rats protected retinal neurons against ischemic insult resulted from an increase in the intra ocular pressure [96]. Data also show that Parawixin II blocked seizures induced by the injection of GABAergic antagonists, bicuculline [99], pentylenetetrazole (PTZ) and picrotoxin, as well as pilocarpine and kainic acid [100]. It is worth noting that the acute injection of Parawixin II does not alter rat behavior in the open field and repeated central injection does not impair acquisition and learning in the Morris water maze. Finally, Parawixin II induces ataxia in the rotarod in doses far higher than effective doses, indicating good therapeutic indexes [100].

Also from South America, the Chilean giant pink tarantula *Grammostola spatulata* paralyzes its preys by injecting a mixture of toxins that blocks ion channels [101]. w-Grammotoxin SIA was isolated from the venom of *G. spatulata* and the potent blocking effect over N-, P-, and Q-type but not L-type of voltage gated calcium channels was reported [102]. The antagonistic activity of w-grammotoxin over voltage dependent calcium channels is considered a therapeutic option to be used in neurodegenerative disorders such as ischemia.

The African tarantula *Hysterocrates gigas* known as the giant baboon spider, inhabits the rain forests of West Africa. The isolation of the venom of *H. gigas*, resulted in the identification of the peptide SNX-482 that blocks R-type voltage dependent calcium channels [103].

The arboreal tarantula *Psalmopoeus cambridgei* is an aggressive spider that lives in the tropical forests of Trindad. As mentioned before, PcTx-1 (π-theraphotoxin-Pc1a) present in *P. cambridgei* venom is the only gating modifier of ASICs [50]. In addition to pain inhibitor, it exerts an interesting neuroprotective and a possible antidepressant activity due to the involvement of ASICs in cell excitability. A drop in pH from neutral 7.4 to more acid extracellular environments, lead to opening of ASICs Na^+ or Ca^{2+} permeable pore, membrane depolarization and increase in Ca^{2+} intracellular concentration [104].

In the light of these facts, Yang and coworkers [105] investigated the neuroprotective activity of PcTx in neurons from newborn piglets submitted to a model of asphyxia-induced cardiac arrest. Data show that the administration of PcTx before the hypoxia-ischemia insult partially prevents the death of neurons in putamen, the most vulnerable encephalic area in this model. The addition of MK-801, a NMDA antagonist, in combination with PcTx exerted better results in cell survival, but in low doses of MK-801. In addition to protection of neuronal cells, treatment with PcTx accelerated neurologic recovery. These results point PcTx as a very unique neurotoxin that should be used as tool in the investigation of processes underlying neuroprotection as well as the design of novel neuroprotective agents.

Bees and wasps are part of the group of the insects, whose stings release a cocktail of toxins, including enzymes, peptides and biogenic amines [106]. Toxins in bee venom have received attention for their properties as anti-inflammatory agents, and in many countries, physicians even prescribe bee stings as treatment of rheumatologic diseases. Recently, Doo and colleagues [107] showed that the bee venom when injected in rats with induced Parkinson disease prevent dopamine neurons cell death, possibly by the inhibition of Jun activation.

Regarding solitary wasps, the most studied wasp species is the European beewolf, *Philanthus triangulum*, the natural predators of honeybees. The adult individuals of this species are herbivores, whereas the larvae eat the paralyzed bees brought to the colony by foraging wasps. The isolation of venom contents begun in the early 80s and revealed that among other classes of molecules, *P. triangulum* venom contains potent acylpolyamines [86]. Philanthotoxins, like other acylpolyamines are mostly potent and selective antagonists of vertebrate and invertebrate glutamate receptors, particularly AMPA receptors [108]. The first isolated and most studied philathotoxin is PhTX-433 and its synthetic analogue, PhTx-343, which antagonize Ach and glutamate ionotropic receptors. The neuroprotective activity of PhTx-343 was tested in cerebellar granule cells culture challenged with NMDA and kainate toxicity and compared to that of Arg-636 [109]. Data showed that both polyamines protected cultures against damage, but Arg-636 was found to be less potent than PhTx-343 against kainate-induced damage. The structural change in PhTx-343 increased its potency, but in higher doses, toxic side effects, were observed.

Due to their lack of selectivity, the use of philanthotoxins as pharmaceuticals may have been limited, and so many modified synthetic analogues were designed for medical treatment

purposes, so far [82]. However, the use of philanthotoxins and other polyamines as tools in research investigation has aided the understanding of several synaptic mechanisms. As it has been recently shown using Ca^{2+}-permeable AMPA receptors expressed in HEK cells. According to [110] the block of these AMPA receptors by PhTx-74, a synthetic analogue of PhTX-433 will reflect structural and biophysical parameters of the channel, such as its subunit composition and mean conductance, respectively. In addition, the investigation of the antagonistic activity of PhTx-343 over ACh receptors showed that the interaction of the toxin with nicotinic receptors is largely voltage dependent, slow and uncompetitive, a similar mode by which they block glutamate ionotropic channels [111].

Going further on wasp venoms, the anticonvulsant and/or neuroprotective effects of molecules in the venom of two Brazilian social species of the genus *Polybia*, were investigated. According to Cunha and co-workers [112] and Mortari and co-workers [113], the non-enzymatic fraction of the venoms of *Polybia ignobilis* and *Polybia occidentalis* inhibit seizures evoked by the injection of several chemoconvulsants in Wistar rats. The neuroactive molecules present in the venom of *P. ignobilis* and *P. occidentalis* are now in phase of structure-function investigation.

Finally, neurotoxins from scorpion venoms have been subject of a wide range of works, mostly approaching the identification of voltage-dependent ion channel activators/blockers. The neuroprotective and/or anticonvulsant activity of these peptides, in turn have received a few lines of investigation [3] despite the ancient use of these animals whole or parts, in the popular medicine in oriental countries, like China [20]. One of the most studied species is the Asian scorpio*n Buthus martensi Karsch* whose venom has several neuroactive peptides, among whose, we may find BmK AEP, which was the first anticonvulsant peptide isolated from scorpion venoms. According to [28], the injection of BmK AEP blocked seizures induced by the injection of coriaria lactone in doses causing no visible side effects [114]. Further isolation of venom of *B. martensi* led to the identification of other peptides, such as BmK AS and BmK Ts and other mostly with analgesic activity. According to Zhao and co-workers [115] BmK AS, a sodium channel modulator at site-4 receptor, inhibited PTZ induced behavioral and electroencephalographic seizures and decreased mean score of pilocarpine-induced seizures. Moreover, these authors showed that BmK AS does not impair locomotion or motor behavior.

The venom of the Mexican scorpion *Centruroides limpidus limpidus*, was fractionated and many activators of voltage-gated ion channel ligands were identified [116]. An exception is Cll9, which stands for *Centruroides limpidus limpidus* toxin nr 9. Cll9 is a 63-residue peptide that has a divergent mode of action; it inhibits sodium channels in superior cervical ganglion neurons and [117]. When injected in Wistar rats via i.c.v., Cll9 inhibited behavioral and electroencephalographic seizures evoked by the microinjection of penicillin into the basolateral amygdala. It is worth noticing that Cll9 has no effect on arthropods such as crickets or crayfish like many sodium channels modulators found in scorpion venoms.

5. Actions on mood disorders

According to the World Health Organization, depression, one of the most important mood disorders, affects up to 5-10% of people worldwide at any time of their lives. Patients with a diagnosed mood disorder are more likely to be women, in productive years, 20 to 40 year-old, and will need in most cases, psychotherapy and/or pharmacological intervention. The costs of these psychiatric and/or psychological disorders are immense, since they affect people regardless of education or socioeconomic status, accounting in the worse cases, for a huge number of suicides. In the United States up to 95% of all suicides, involve mentally ill people, accounting for 1.3% of all deaths [118]. A recent survey shows that generalized anxiety disorder, posttraumatic stress disorder, social anxiety disorder and panic disorder are highly predictive of suicidal idealization [118]. Many aspects of the pathophysiology of mood disorders, as well as the regulation of normal mood states remain unknown. However, with the improvement of techniques for research and diagnosis, such as positron emission tomography, magnetic resonance and multiple channels recording electroencephalogram, soon researchers will be capable to identify structures and neurochemical mechanism involved in the regulation of mental states, including mood. So far, we know that limbic structures, such as the amygdala, hippocampus, hypothalamus and prefrontal cortex control the emotional aspects of brain function. There are plenty of connections among these structures, which might be involved in the onset of mood disorders [119].

Pharmacological treatment of mood disorders consists in daily intake of anti-depressants, anxiolytics or anti-psychotics, most of which cause a wide set of undesired side effects that impose restrictions to patients quality of life. In this regard novel drugs prescribed for mood disorder, such as serotonin uptake inhibitors might be better tolerated and safer than classical drugs, such as monoamine oxidase inhibitors. Among the observed undesired effects we can cite; dizziness, sedation, sexual dysfunction and suicidal though, a paradoxical effect of serotonin uptake inhibitors [119]. Aside from tolerability, medicines used as treatment of depression for example, take too long to produce effect in only a minority of patients; 35-45% of treated patients [120]. Therefore, there is a still great need for novel alternatives to be used both, in basic and clinical science.

The neurochemistry of mood disorders is complex and there is a list of candidates for targets of mood stabilizers, such as adrenaline, GABA, serotonin and glutamate receptors and transporters. There is not many works relating neurotoxins from arthropods and mood disorders, but the few works available showed that in some cases, these molecules might contribute for the development of novel drugs.

The venom of the Brazilian colonial spider *Parawixia bistriata* was fractionated and tested in many animal models of epilepsy, neurodegeneration and anxiety. According to [121], the microinjection of Parawixin 2 (formerly FrPbAII) in the dorsal hippocampus of male Wistar rats increased the time spent in the open arms of the elevated plus maze. Moreover, rats exposed to the light-dark choice apparatus spent more time in the light side of the box, similarly to what observed for diazepam or nipecotic acid, a GABA transporter-1 (GAT-1) inhibitor [121]. In another investigation of *P. bistriata* venom contents, Saidemberg and co-workers

[122] isolated PwTx-I and tested the inhibitory activity of this neurotoxin and its enantiomers on mammalian monoamine oxidases (MAO)-A and -B. According to these authors, PwTx-I, acted as non-competitive inhibitors of MAO-A and MAO-B. MAO metabolizes monoamines dopamine, serotonin and adrenaline, terminating monoaminergic transmission. Inhibitors or MAO (MAOi) have been extensively used as mood stabilizers and currently they have received attention due to their protective activity against age-induced neurodegenerative disorders [123].

Considering alternative targets for mood stabilizers, interesting results were obtained with PcTx, isolated from the venom of the spider *P. cambridgei*, a selective blocker of ASICs. Data showed that both PcTx and amiloride attenuated the stress-induced hyperthermia, whereas only the administration of PcTx increased number of punished crosses measured in the four-plate test. These results indicate that both blockers could attenuate autonomic anxiety parameters, but only PcTx exerted effects on the behavioral anxiety parameters [124].

The aggressive Brazilian social wasp *Agelaia vicina*, builds huge nests where with over a million of individuals. The neurobiological activity of the venom of *A. vicina*, was investigated. Oliveira and colleagues [125] showed that the central injection of the non-enzymatic fraction of the venom induced catalepsy in Wistar compared to the neuroleptic drug haloperidol, a nonselective D2 dopamine antagonist. This effect was reversed by the injection of theophylline or ketamine. The fractionation of the venom led to the identification of two peptides, AvTx-7, mastoparan, and AvTx-8. The investigation of AvTx-8 mode of action in vivo, was performed in a model of panic induction through the activation of GABAergic pathways connecting mesencephalic substantia nigra pars reticulate to superior colliculus [126]. These experiments showed that intranigral microinjection of AvTx-8 inhibited the panic like response induced by the GABAergic blockade of superior colliculus. These effects were similar to those of baclofen, a $GABA_B$ agonist, but differed from the effects of muscimol, a $GABA_A$ agonist. Since post-synaptic $GABA_B$ is a metabotropic receptor complex with a potassium channel, AvTx-8 could act in many different sites that would end in channels opening and hyperpolarization of neuronal membrane.

6. Tools for the study of the functioning of the CNS: learning and memory

Neurotoxins isolated from arthropod are important tools to study of the normal function of the CNS, especially in the structure-function research of the ion channels and the interaction the blockers and modulators in the regulation of the learning and memory (for revision see [127]). In this context, the principal compound used in study of the mechanism of the learning and memory in models of experimental animals is the apamin. Apamin is a short peptide (18 aa) isolated from the venom of honeybee, *Apis mellifera*. It is generally accepted that apamin selectively blocks small conductance calcium-activated potassium channels (SK or K_{ca}), although evidences point to an allosteric modulation of opening rather than the block of the pore [128]. Upon an increase in intracellular calcium, SK channels will open and allow

an outward current of potassium ions that is responsible for the hyperpolarization phase of the action potentials. Most studies on structure-function of SK channels were conducted using apamin blockade. The homomeric or heteromeric expression of these channels occurs in higher brain areas such as the neocortex, hippocampus and sub-cortical areas such as thalamus and basal ganglia as well as in cerebellum and brainstem. Substantial data SK channels show the involvement of SK channels in processes of learning and memory, and apamin blockade of SK lead to an increase in cellular excitability, facilitates synaptic plasticity and memory processes run by the hippocampus. In addition, apamin induces alterations in dendritic morphology that might counteract aging and neurodegenerative processes that lead to cognitive and memory impairment [129]. In fact, SK channels co-localize with Ca^{2+}-permeable NMDA receptors in the CA1 region of the hippocampus and the entry of calcium in the cell through these receptors might activate SK that will hyperpolarize membrane. The blockade of SK channels will modulate hippocampal excitability that is essential in memory processes such as long-term potentiation a commonly observed event of synaptic plasticity. Due to its actions, the use of apamin as a tool in research has been consolidated. In addition, the therapeutic use of apamin, in order to maintain hippocampal function and avoid the deleterious effects of aging in memory and cognitive processes have also been proposed [129].

Besides apamin, modulators peptides of the potassium channel isolated from scorpion also have been tested in models of the learning and memory. The good examples are: Charybdotoxin isolated from scorpion *Leiurus quinquestriatus*, Kaliotoxin isolated from *Androctonus mauretanicus* and Iberiotoxin from *Buthus tasmulus*. Charybdotoxin is a potent selective inhibitor of high (large or big) conductance Ca^{2+}-activated potassium channels (KCa1.1, BK, or maxi-K), as well as a Kv1.3 channel [130]. Kaliotoxin is a specific inhibitor of Kv1.1 and Kv1.3 [131] and Iberiotoxin is a selective inhibitor of KCa1.1 channels (formerly BK) [132]. These peptides induced an improvement effect in passive avoidance test and olfactory discrimination task [133,134].

7. Final remarks

The stories of voltage-gated, ligand-gated ion channels and venom toxins are very closely tied. Indeed, the isolation and structural characterization of venom molecules provided a plethora of tools that have been used in the investigation of ion channels structure-function relationships. With the aid of arthropod toxins, remarkably, scorpionic toxins, the characterization of sodium channels was possible. Spider and wasps polyamines, in turn are considered unique ligands of glutamatergic and cholinergic ionotropic receptors. Regarding to peptides and small proteins, arthropod venoms possess an arsenal of these molecules that remain largely unknown and consequently, their pharmacological potential is left unexplored.

Due to the mode of action of neurotoxins, their affinity and selectivity for neuronal structures, many researchers consider them as probes to novel drugs design and development. However, despite of the thousands of patents made with neurotoxins in the past 30 years, very few molecules came to commercialization.

Acknowledgements

Author details

Márcia Renata Mortari[1*] and Alexandra Olimpio Siqueira Cunha[2]

*Address all correspondence to: mmortari@unb.br

1 Department of Physiological Sciences, Institute of Biological Sciences, University of Brasília, Brazil

2 Department of Physiology, FMRP, University of São Paulo, Brazil

References

[1] Zhijian, C., Chao, D., Dahe, J., & Wenxin, L. (2006). The effect of intron location on the splicing of BmKK2 in 293T cells. *J Biochem Mol Toxicol*, 20(3), 127-32.

[2] Estrada, G., Villegas, E., & Corzo, G. (2007). Spider venoms: a rich source of acylpolyamines and peptides as new leads for CNS drugs. *Nat Prod Rep*, 24(1), 145-61.

[3] Mortari, M. R., Cunha, A. O., Ferreira, L. B., & dos, Santos. W. F. (2007). Neurotoxins from invertebrates as anticonvulsants: from basic research to therapeutic application. *Pharmacol Ther.*, 114(2), 171-83.

[4] Rogoza, L. N., Salakhutdinov, N. F., & Tolstikov, G. A. (2005). Polymethyleneamine alkaloids of animal origin. I. Metabolites of marine and microbial organisms]. *Bioorg Khim*, 31(6), 563-77.

[5] WHO. (2007). Neurological Disorders:. *Public Health Challenges*.

[6] Bialer, M., & White, H. S. (2010). Key factors in the discovery and development of new antiepileptic drugs. *Nature reviews Drug discovery*, 9(1), 68-82.

[7] Blier, P. (2010). The well of novel antidepressants: running dry. *J Psychiatry Neurosci*, 35(4), 219-20.

[8] Goudet, C., Chi, C. W., & Tytgat, J. (2002). An overview of toxins and genes from the venom of the Asian scorpion Buthus martensi Karsch. *Toxicon official journal of the International Society on Toxinology*, 40(9), 1239-58.

[9] Cherniack, E. P. (2011). Bugs as drugs, part two: worms, leeches, scorpions, snails, ticks, centipedes, and spiders. *Alternative medicine review : a journal of clinical therapeutic*, 16(1), 50-8.

[10] Ratcliffe, N. A., Mello, C. B., Garcia, E. S., Butt, T. M., & Azambuja, P. (2011). Insect natural products and processes: new treatments for human disease. *Insect Biochem Mol Biol*, 41(10), 747-69.

[11] Monteiro, M. C., Romao, P. R., & Soares, A. M. (2009). Pharmacological perspectives of wasp venom. *Protein and peptide letters*, 16(8), 944-52.

[12] Beleboni, R. D., Pizzo, A. B., Fontana, A. C. K., Carolino, R. D. O. G., Coutinho-Netto, J., & dos, Santos. W. F. (2004). Spider and wasp neurotoxins: pharmacological and biochemical aspects. *Eur J Pharmacol*, 493(1-3), 1-17.

[13] Ashburn, M. A., & Staats, P. S. (1999). Management of chronic pain. *Lancet*, 353(167), 865-869.

[14] Stucky, C. L., Gold, M. S., & Zhang, X. (2001). Mechanisms of pain. *Proc Natl Acad Sci U S A.*, 98(21), 11845-6.

[15] Sindrup, S. H., & Jensen, T. S. (1999). Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. *Pain*, 83(3), 389-400.

[16] Villetti, G., Bergamaschi, M., Bassani, F., Bolzoni, P. T., Maiorino, M., Pietra, C., et al. (2003). Antinociceptive activity of the N-methyl-D-aspartate receptor antagonist N-(2-Indanyl)-glycinamide hydrochloride (CHF3381) in experimental models of inflammatory and neuropathic pain. *J Pharmacol Exp Ther*, 306(2), 804-14.

[17] Ji, Y. H., Mansuelle, P., Terakawa, S., Kopeyan, C., Yanaihara, N., Hsu, K., et al. (1996). Two neurotoxins (BmK I and BmK II) from the venom of the scorpion Buthus martensi Karsch: purification, amino acid sequences and assessment of specific activity. *Toxicon : official journal of the International Society on Toxinology*, 34(9), 987-1001.

[18] Guan, R., Wang, C. G., Wang, M., & Wang, D. C. (2001). A depressant insect toxin with a novel analgesic effect from scorpion Buthus martensii Karsch. *Biochim Biophys Acta*, 1549(1), 9-18.

[19] Rodriguez dela Vega, R. C., & Possani, L. D. (2005). Overview of scorpion toxins specific for channels and related peptides: biodiversity, structure-function relationships and evolution. *Toxicon : official journal of the International Society on Toxinology*, 46(8), 831-44.

[20] Xiong, Y. M., Lan, Z. D., Wang, M., Liu, B., Liu, X. Q., Fei, H., et al. (1999). Molecular characterization of a new excitatory insect neurotoxin with an analgesic effect on mice from the scorpion Buthus martensi Karsch. *Toxicon*, 37(8), 1165-80.

[21] Escoubas, P., Stankiewicz, M., Takaoka, T., Pelhate, M., Romi-Lebrun, R., Wu, F. Q., et al. (2000). Sequence and electrophysiological characterization of two insect-selec-

tive excitatory toxins from the venom of the Chinese scorpion Buthus martensi. *FEBS Lett*, 483(2-3), 175 -80 .

[22] Guan, R. J., Wang, M., Wang, D., & Wang, D. C. (2001). A new insect neurotoxin AngP1 with analgesic effect from the scorpion Buthus martensii Karsch: purification and characterization. *The journal of peptide research official journal of the American Peptide Society*, 58(1), 27-35.

[23] Wang, C. Y., Tan, Z. Y., Chen, B., Zhao, Z. Q., & Ji, Y. H. (2000). Antihyperalgesia effect of BmK IT2, a depressant insect-selective scorpion toxin in rat by peripheral administration. *Brain research bulletin*, 53(3), 335-8.

[24] Tan, Z. Y., Xiao, H., Mao, X., Wang, C. Y., Zhao, Z. Q., & Ji, Y. H. (2001). The inhibitory effects of BmK IT2, a scorpion neurotoxin on rat nociceptive flexion reflex and a possible mechanism for modulating voltage-gated Na(+) channels. *Neuropharmacology*, 40(3), 352-7.

[25] Bai, Z. T., Liu, T., Pang, X. Y., Chai, Z. F., & Ji, Y. H. (2007). Suppression by intrathecal BmK IT2 on rat spontaneous pain behaviors and spinal c-Fos expression induced by formalin. *Brain Res Bull*, 73(4-6), 248 -253 .

[26] Zhang, X. Y., Bai, Z. T., Chai, Z. F., Zhang, J. W., Liu, Y., & Ji, Y. H. (2003). Suppressive effects of BmK IT2 on nociceptive behavior and c-Fos expression in spinal cord induced by formalin. *J Neurosci Res*, 74(1), 167-73.

[27] Li, Y. J., Tan, Z. Y., & Ji, Y. H. (2000). The binding of BmK IT2, a depressant insect-selective scorpion toxin on mammal and insect sodium channels. *Neurosci Res*, 38(3), 257-64.

[28] Wang, Y., Wang, L., Cui, Y., Song, Y. B., Liu, Y. F., Zhang, R., et al. (2011). Purification, characterization and functional expression of a new peptide with an analgesic effect from Chinese scorpion Buthus martensii Karsch (BmK AGP-SYPU1). *Biomed Chromatogr*, 25(7), 801-7.

[29] Ji-H, Y., Li-J, Y., Zhang-W, J., Song-L, B., Yamaki, T., Mochizuki, T., et al. (1999). Covalent structures of BmK AS and BmK AS-1, two novel bioactive polypeptides purified from Chinese scorpion Buthus martensi Karsch. *Toxicon*, 37(3), 519-36.

[30] Lan-D, Z., Dai, L., Zhuo-L, X., Feng-C, J., Xu, K., & Chi-W, C. (1999). Gene cloning and sequencing of BmK AS and BmK AS-1, two novel neurotoxins from the scorpion Buthus martensi Karsch. *Toxicon*, 37(5), 815-23.

[31] Chen, B., & Ji, Y. (2002). Antihyperalgesia effect of BmK AS, a scorpion toxin, in rat by intraplantar injection. *Brain Res*, 952(2), 2322-6.

[32] Chen, J., Feng, X. H., Shi, J., Tan, Z. Y., Bai, Z. T., Liu, T., et al. (2006). The anti-nociceptive effect of BmK AS, a scorpion active polypeptide, and the possible mechanism on specifically modulating voltage-gated Na+ currents in primary afferent neurons. *Peptides*, 27(9), 182-92.

[33] Tan, Z. Y., Mao, X., Xiao, H., Zhao, Z. Q., & Ji, Y. H. (2001). Buthus martensi Karsch agonist of skeletal-muscle RyR-1, a scorpion active polypeptide: antinociceptive effect on rat peripheral nervous system and spinal cord, and inhibition of voltage-gated Na(+) currents in dorsal root ganglion neurons. *Neurosci Lett*, 297(2), 65-8.

[34] Wu, Y., Ji, Y. H., & Shi, Y. L. (2001). Sodium current in NG108-15 cell inhibited by scorpion toxin BmKAS-1 and restored by its specific monoclonal antibodies. *J Nat Toxins*, 10(3), 193-8.

[35] Liu, Y. F., Ma, R. L., Wang, S. L., Duan, Z. Y., Zhang, J. H., Wu, L. J., et al. (2003). Expression of an antitumor-analgesic peptide from the venom of Chinese scorpion Buthus martensii karsch in Escherichia coli. *Protein Expr Purif*, 27(2), 253-8.

[36] Cui, Y., Guo, G. L., Ma, L., Hu, N., Song, Y. B., Liu, Y. F., et al. (2010). Structure and function relationship of toxin from Chinese scorpion Buthus martensii Karsch (BmKAGAP): gaining insight into related sites of analgesic activity. *Peptides*, 31(6), 995-1000.

[37] Cao, Z. Y., Mi, Z. M., Cheng, G. F., Shen, W. Q., Xiao, X., Liu, X. M., et al. (2004). Purification and characterization of a new peptide with analgesic effect from the scorpion Buthus martensi Karch. *J Pept Res*, 64(1), 33-41.

[38] Peng, K., Chen, X. D., & Liang, S. P. (2001). The effect of Huwentoxin-I on Ca(2+) channels in differentiated NG108-15 cells, a patch-clamp study. *Toxicon : official journal of the International Society on Toxinology*, 39(4), 491-8.

[39] Chen, J. Q., Zhang, Y. Q., Dai, J., Luo, Z. M., & Liang, S. P. (2005). Antinociceptive effects of intrathecally administered huwentoxin-I, a selective N-type calcium channel blocker, in the formalin test in conscious rats. *Toxicon : official journal of the International Society on Toxinology*, 45(1), 15-20.

[40] Wen, Tao. Z., Gu, Yang. T., Ying, R., Mao, Cai. W., Lin, L., Chi, Miao. L., et al. (2011). The antinociceptive efficacy of HWTX-I epidurally administered in rheumatoid arthritis rats. *Int J Sports Med*, 32(11), 869-74.

[41] Saez, N. J., Senff, S., Jensen, J. E., Er, S. Y., Herzig, V., Rash, L. D., et al. (2010). Spider-venom peptides as therapeutics. *Toxins*, 2(12), 2851-71.

[42] Murakami, M., Nakagawasai, O., Suzuki, T., Mobarakeh, I. I., Sakurada, Y., Murata, A., et al. (2004). Antinociceptive effect of different types of calcium channel inhibitors and the distribution of various calcium channel alpha 1 subunits in the dorsal horn of spinal cord in mice. *Brain Res*, 1024(1-2), 122 -9.

[43] Rajendra, W., Armugam, A., & Jeyaseelan, K. (2004). Toxins in anti-nociception and anti-inflammation. *Toxiconofficial journal of the International Society on Toxinology*, 44(1), 1-17.

[44] Souza, A. H., Ferreira, J., Cordeiro, Mdo. N., Vieira, L. B., De Castro, C. J., Trevisan, G., et al. (2008). Analgesic effect in rodents of native and recombinant Ph alpha 1beta

toxin, a high-voltage-activated calcium channel blocker isolated from armed spider venom. *Pain*, 140(1), 115-26.

[45] Dalmolin, G. D., Silva, C. R., Rigo, F. K., Gomes, G. M., Cordeiro, Mdo. N., Richardson, M., et al. (2011). Antinociceptive effect of Brazilian armed spider venom toxin Tx3-3 in animal models of neuropathic pain. *Pain*, 152(10), 2224-32.

[46] Vieira, L. B., Kushmerick, C., Hildebrand, M. E., Garcia, E., Stea, A., Cordeiro, M. N., et al. (2005). Inhibition of high voltage-activated calcium channels by spider toxin PnTx3-6. *J Pharmacol Exp Ther*, 314(3), 1370-7.

[47] Prado, MA, Guatimosim, C., Gomez, M. V., Diniz, C. R., Cordeiro, M. N., & Romano-Silva, MA. (1996). A novel tool for the investigation of glutamate release from rat cerebrocortical synaptosomes: the toxin Tx from the venom of the spider Phoneutria nigriventer. *Biochem J*, 314(Pt1), 145-50.

[48] De Souza, A. H., Lima, M. C., Drewes, C. C., da, Silva. J. F., Torres, K. C., Pereira, E. M., et al. (2011). Antiallodynic effect and side effects of Phalpha1beta, a neurotoxin from the spider Phoneutria nigriventer: comparison with omega-conotoxin MVIIA and morphine. *Toxicon : official journal of the International Society on Toxinology*, 58(8), 626-33.

[49] Malmberg, A. B., & Yaksh, T. L. (1994). Voltage-sensitive calcium channels in spinal nociceptive processing: blockade of N- and P-type channels inhibits formalin-induced nociception. *J Neurosci*, 14(8), 4882-90.

[50] Saez, N. J., Mobli, M., Bieri, M., Chassagnon, I. R., Malde, A. K., Gamsjaeger, R., et al. (2011). A dynamic pharmacophore drives the interaction between Psalmotoxin-1 and the putative drug target acid-sensing ion channel 1a. Mol Pharmacol Epub 2011/08/10., 80(5), 796-808.

[51] Mc Cleskey, E. W., & Gold, M. S. (1999). Ion channels of nociception. *Annu Rev Physiol*, 61, 835-56.

[52] Waldmann, R., Champigny, G., Lingueglia, E., De Weille, J. R., Heurteaux, C., & Lazdunski, M. (1999). H(+)-gated cation channels. *Ann N Y Acad Sci*, 868, 67-76.

[53] Mazzuca, M., Heurteaux, C., Alloui, A., Diochot, S., Baron, A., Voilley, N., et al. (2007). A tarantula peptide against pain via ASIC1a channels and opioid mechanisms. *Nat Neurosci*, 10(8), 943-5.

[54] Pignataro, G., Simon, R. P., & Xiong, Z. G. (2007). Prolonged activation of ASIC1a and the time window for neuroprotection in cerebral ischaemia. *Braina journal of neurology*, 130(Pt 1), 151 -158 .

[55] Lampe, R. A. (1999). Analgesic peptides from venom of grammostola spatulata and use thereof. http://www.patentgenius.com/patent/5776896.html.

[56] Grishin, E. V., Savchenko, G. A., Vassilevski, A. A., Korolkova, Y. V., Boychuk, Y. A., Viatchenko-Karpinski, V. Y., et al. (2010). Novel peptide from spider venom inhibits 2X3receptors and inflammatory pain. *Ann Neurol*, 67(5), 680-3.

[57] Chen, J., & Lariviere, W. R. (2010). The nociceptive and anti-nociceptive effects of bee venom injection and therapy: a double-edged sword. *Prog Neurobiol*, 92(2), 151-83.

[58] Kwon, Y. B., Kang, M. S., Kim, H. W., Ham, T. W., Yim, Y. K., Jeong, S. H., et al. (2001). Antinociceptive effects of bee venom acupuncture (apipuncture) in rodent animal models: a comparative study of acupoint versus non-acupoint stimulation. *Acupunct Electrother Res*, 26(1-2), 59-68.

[59] Kwon, Y. B., Kim, J. H., Yoon, J. H., Lee, JD, Han, H. J., Mar, W. C., et al. (2001). The analgesic efficacy of bee venom acupuncture for knee osteoarthritis: a comparative study with needle acupuncture. *Am J Chin Med*, 29(2), 187-99.

[60] Lee-H, J., Kwon-B, Y., Han-J, H., Mar-C, W., Lee-J, H., Yang-S, I., et al. (2001). Bee Venom Pretreatment Has Both an Antinociceptive and Anti-Inflammatory Effect on Carrageenan-Induced Inflammation. *J Vet Med Sci*, 63(3), 251-9.

[61] Lee, J. D., Park, H. J., Chae, Y., & Lim, S. (2005). An Overview of Bee Venom Acupuncture in the Treatment of Arthritis. *Evidence-based complementary and alternative medicine : eCAM*, 2(1), 79-84.

[62] Kwon, Y. B., Kang, M. S., Han, H. J., Beitz, A. J., & Lee, J. H. (2001). Visceral antinociception produced by bee venom stimulation of the Zhongwan acupuncture point in mice: role of alpha(2) adrenoceptors. *Neurosci Lett*, 308(2), 133-7.

[63] Baek, Y. H., Huh, J. E., Lee, J. D., Choi do, Y., & Park, D. S. (2006). Antinociceptive effect and the mechanism of bee venom acupuncture (Apipuncture) on inflammatory pain in the rat model of collagen-induced arthritis: Mediation by alpha2-Adrenoceptors. *Brain Res*, 073-074, 305-10.

[64] Son, D. J., Kang, J., Kim, T. J., Song, H. S., Sung, K. J., Yun, Y., et al. (2007). Melittin, a major bioactive component of bee venom toxin, inhibits PDGF receptor beta-tyrosine phosphorylation and downstream intracellular signal transduction in rat aortic vascular smooth muscle cells. *Journal of toxicology and environmental health Part A*, 70(15-16), 1350 -5 .

[65] Lauterwein, J., Bosch, C., Brown, L. R., & Wuthrich, K. (1979). Physicochemical studies of the protein-lipid interactions in melittin-containing micelles. *Biochim Biophys Acta*, 556(2), 244-64.

[66] Lavialle, F., Levin, I. W., & Mollay, C. (1980). Interaction of melittin with dimyristoyl phosphatidylcholine liposomes: evidence for boundary lipid by Raman spectroscopy. *Biochim Biophys Acta*, 600(1), 62-71.

[67] Shier WT. (1979). Activation of high levels of endogenous phospholipase A2 in cultured cells. *Proc Natl Acad Sci U S A*, 76(1), 195-9.

[68] Lad, P. J., & Shier, W. T. (1979). Activation of microsomal guanylate cyclase by a cytotoxic polypeptide: melittin. *Biochem Biophys Res Commun*, 89(1), 315-21.

[69] Park, H. J., Lee, H. J., Choi, M. S., Son, D. J., Song, H. S., Song, M. J., et al. (2008). JNK pathway is involved in the inhibition of inflammatory target gene expression and NF-kappaB activation by melittin. *J Inflamm (Lond).*, 5, 7.

[70] Merlo, L. A., Bastos, L. F., Godin, A. M., Rocha, L. T., Nascimento, E. B. Jr, Paiva, A. L., et al. (2011). Effects induced by Apis mellifera venom and its components in experimental models of nociceptive and inflammatory pain. *Toxicon : official journal of the International Society on Toxinology*, 57(5), 764-71.

[71] Shkenderov, S., & Koburova, K. (1982). Adolapin- A newly isolated analgetic and anti-inflammatory polypeptide from bee venom. *Toxicon*, 20(1), 317-21.

[72] Koburova, K. L. S. G., & Michailova, S. V. (1985). Shkenderov Further investigation on the antiinflammatory properties of adolapin--bee venom polypeptide. *Acta Physiol Pharmacol Bulg*, 11(2), 50-5.

[73] Mortari, M. R., Cunha, A. O., Carolino, R. O., Coutinho-Netto, J., Tomaz, J. C., Lopes, N. P., et al. (2007). Inhibition of acute nociceptive responses in rats after i.c.v. injection of Thr6-bradykinin, isolated from the venom of the social wasp, Polybia occidentalis. *Br J Pharmacol*, 151(6), 860-9.

[74] Pellegrini, M., Mammi, S., Peggion, E., & Mierke, D. F. (1997). Threonine6-bradykinin: structural characterization in the presence of micelles by nuclear magnetic resonance and distance geometry. *J Med Chem*, 40(1), 92-8.

[75] Pellegrini, M., & Mierke, D. F. (1997). Threonine6-bradykinin: molecular dynamics simulations in a biphasic membrane mimetic. *J Med Chem*, 40(1), 99-104.

[76] Nagy, I., Paule, C., White, J., & Urban, L. (2007). Taking the sting out of pain. *Br J Pharmacol*, 151(6), 721-2.

[77] Kawai, N., Shimazaki, K., Sahara, Y., Robinson, H. P., & Nakajima, T. (1991). Spider toxin and the glutamate receptors. *Comp Biochem Physiol C*, 98(1), 87-95.

[78] Sorkin, L. S. Y. T., & Doom, C. M. (1999). Mechanical allodynia in rats is blocked by a Ca2+ permeable AMPA receptor antagonist. *Neuroreport*, 10(17), 3523-6.

[79] Stanfa, L. C., Hampton, D. W., & Dickenson, A. H. (2000). Role of Ca2+-permeable non-NMDA glutamate receptors in spinal nociceptive transmission. Neuroreport Epub 2000/10/24., 11(14), 3199-202.

[80] Jones, M. G., & Lodge, D. (1991). Comparison of some arthropod toxins and toxin fragments as antagonists of excitatory amino acid-induced excitation of rat spinal neurones. *Eur J Pharmacol*, 204(2), 203-9.

[81] Sorkin, L. S., Yaksh, T. L., & Doom, C. M. (2001). Pain models display differential sensitivity to Ca2+-permeable non-NMDA glutamate receptor antagonists. *Anesthesiology*, 95(4), 965-73.

[82] Andersen, T. F., Vogensen, S. B., Jensen, L. S., Knapp, K. M., & Stromgaard, K. (2005). Design and synthesis of labeled analogs of PhTX-56, a potent and selective AMPA receptor antagonist. *Bioorg Med Chem*, 13(17), 5104-12.

[83] Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacol Rev*, 51(1), 7-61.

[84] Rajendra, W., Armugam, A., & Jeyaseelan, K. (2004). Neuroprotection and peptide toxins. *Brain Res Brain Res Rev*, 45(2), 125-41.

[85] Grishin, E. V., Volkova, T. M., & Arseniev, A. S. (1989). Isolation and structure analysis of components from venom of the spider Argiope lobata. *Toxicon*, 27(5), 541-9.

[86] Clark, R. B., Donaldson, P. L., Gration, K. A., Lambert, J. J., Piek, T., Ramsey, R., et al. (1982). Block of locust muscle glutamate receptors by delta-philanthotoxin occurs after receptor activations. *Brain Res*, 241(1), 105-14.

[87] Usherwood, P. N., & Blagbrough, I. S. (1991). Spider toxins affecting glutamate receptors: polyamines in therapeutic neurochemistry. Pharmacol Ther Epub 1991/11/01., 52(2), 245-68.

[88] Herold, E. E., & Yaksh, T. L. (1992). Anesthesia and muscle relaxation with intrathecal injections of AR636 and AG489, two acylpolyamine spider toxins, in rat. *Anesthesiology*, 77(3), 507-12.

[89] Kanai, H., Ishida, N., Nakajima, T., & Kato, N. (1992). An analogue of Joro spider toxin selectively suppresses hippocampal epileptic discharges induced by quisqualate. *Brain Res*, 581(1), 161-4.

[90] Roy, J., Minotti, S., Dong, L., Figlewicz, D. A., & Durham, H. D. (1998). Glutamate potentiates the toxicity of mutant Cu/Zn-superoxide dismutase in motor neurons by postsynaptic calcium-dependent mechanisms. *J Neurosci*, 18(23), 9673-84.

[91] Seymour, P. A. M. E. (1989). In vivo antagonist activity of the polyamine spider venom component, Argiotoxin-636. *Soc Neurosci Abs*, 15, 463-24.

[92] K, W. (1993). Effects of Agelenopsis aperta toxins on the N-methyl-D-aspartate receptor: polyamine-like and high-affinity antagonist actions. *J Pharmacol Exp Ther*, 266(1), 231-6.

[93] Miranda, D. M., Romano-Silva, M. A., Kalapothakis, E., Diniz, C. R., Cordeiro, M. N., Moraes-Santos, T., et al. (2001). Spider neurotoxins block the beta scorpion toxin-induced calcium uptake in rat brain cortical synaptosomes. *Brain Res Bull*, 54(5), 533-6.

[94] Pinheiro, A. C., da Silva, A. J., Prado Cordeiro, MA, Mdo, N., Richardson, M., Batista, M. C., et al. (2009). Phoneutria spider toxins block ischemia-induced glutamate release, neuronal death, and loss of neurotransmission in hippocampus. *Hippocampus*, 19(11), 1123-9.

[95] Agostini, R. M., Nascimento do Pinheiro, A. C., Binda, N. S., Romano, Silva., Nascimento do, Cordeiro. M., Richardson, M., et al. (2011). Phoneutria spider toxins block

ischemia-induced glutamate release and neuronal death of cell layers of the retina. *Retina*, 31(7), 1392-9.

[96] Beleboni, R. O., Guizzo, R., Fontana, A. C., Pizzo, A. B., Carolino, R. O., Gobbo-Neto, L., et al. (2006). Neurochemical characterization of a neuroprotective compound from Parawixia bistriata spider venom that inhibits synaptosomal uptake of GABA and glycine. *Mol Pharmacol*, 69(6), 1998-2006.

[97] Fontana, A. C., Guizzo, R., de Oliveira, Beleboni. R., Meirelles, E. S. A. R., Coimbra, N. C., Amara, S. G., et al. (2003). Purification of a neuroprotective component of Para-wixia bistriata spider venom that enhances glutamate uptake. *Br J Pharmacol*, 139(7), 1297-309.

[98] Fontana, A. C., de Oliveira, Beleboni. R., Wojewodzic, M. W., Ferreira, Dos., Santos, W., Coutinho-Netto, J., Grutle, N. J., et al. (2007). Enhancing glutamate transport: mechanism of action of Parawixin1, a neuroprotective compound from Parawixia bistriata spider venom. *Mol Pharmacol*, 72(5), 1228-37.

[99] Cairrão, M. A. R. R. A., Pizzo, A. B., Fontana, A. C. K., Beleboni, R. O., Coutinho-Ne-to, J., Miranda, A., & Santos, W. F. (2002). Anticonvulsant and GABA uptake inhibi-tion properties of P. bistriata and S. raptoria spider venom fractions. *Pharm Biol*, 40(6), 472-7.

[100] Gelfuso, E. A., Cunha, A. O., Mortari, M. R., Liberato, J. L., Paraventi, K. H., Beleboni, R. O., et al. (2007). Neuropharmacological profile of FrPbAII, purified from the ven-om of the social spider Parawixia bistriata (Araneae, Araneidae), in Wistar rats. *Life Sci*, 80(6), 566-72.

[101] Lampe, R. A., Defeo, P. A., Davison, Young. J., Herman, J. L., Spreen, R. C., et al. (1993). Isolation and pharmacological characterization of omega-grammotoxin SIA, a novel peptide inhibitor of neuronal voltage-sensitive calcium channel responses. *Mol Pharmacol*, 44(2), 451-60.

[102] Piser, T. M., Lampe, R. A., Keith, R. A., & Thayer, S. A. (1995). Complete and reversi-ble block by ω-grammotoxin SIA of glutamatergic synaptic transmission between cultured rat hippocampal neurons. *Neurosci Lett*, 201(2), 135-8.

[103] Newcomb, R. P. A., Szoke, B. G., Tarczy-Hornoch, K., Hopkins, W. F., Cong, R. L., Miljanich, G. P., Dean, R., Nadasdi, L., Urge, L., & Bowersox, S. S. (2005). inventor; Elan Pharmaceuticals Inc US, assignee. Class e voltage-gated calcium channel antag-onist and methods patent 0920504

[104] Chu, X. P., Papasian, C. J., Wang, . J. Q., & Xiong, Z. G. (2011). Modulation of acid-sensing ion channels: molecular mechanisms and therapeutic potential. *International journal of physiology, pathophysiology and pharmacology*, 3(4), 288-309.

[105] Yang, Z. J., Ni, X., Carter, E. L., Kibler, K., Martin, L. J., & Koehler, R. C. (2011). Neu-roprotective effect of acid-sensing ion channel inhibitor psalmotoxin-1 after hypoxia-ischemia in newborn piglet striatum. *Neurobiol Dis*, 43(2), 446-54.

[106] Libersat, F. (2003). Wasp uses venom cocktail to manipulate the behavior of its cockroach prey. *Journal of comparative physiology A, Neuroethology, sensory, neural, and behavioral physiology*, 189(7), 497-508.

[107] Doo, A. R., Kim, S. T., Kim, S. N., Moon, W., Yin, C. S., Chae, Y., et al. (2010). Neuroprotective effects of bee venom pharmaceutical acupuncture in acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson's disease. *Neurol Res*, 32(1), 88-91.

[108] Stromgaard, K. (2005). Natural products as tools for studies of ligand-gated ion channels. *Chem Rec*, 5(4), 229-39.

[109] Green, A. C., Nakanishi, K., & Usherwood, P. N. (1996). Polyamine amides are neuroprotective in cerebellar granule cell cultures challenged with excitatory amino acids. *Brain Res*, 717(1-2), 135 -46 .

[110] Jackson, A. C., Milstein, A. D., Soto, D., Farrant, M., Cull-Candy, S. G., & Nicoll, R. A. (2011). Probing TARP modulation of AMPA receptor conductance with polyamine toxins. *J Neurosci*, 31(20), 7511-20.

[111] Brier, T. J., Mellor, I. R., Tikhonov, D. B., Neagoe, I., Shao, Z., Brierley, MJ, et al. (2003). Contrasting actions of philanthotoxin-343 and philanthotoxin-(12) on human muscle nicotinic acetylcholine receptors. *Mol Pharmacol*, 64(4), 954-64.

[112] Cunha, A. O., Mortari, M. R., Oliveira, L., Carolino, R. O., Coutinho-Netto, J., & dos Santos, W. F. (2005). Anticonvulsant effects of the wasp Polybia ignobilis venom on chemically induced seizures and action on GABA and glutamate receptors. *Comparative biochemistry and physiology Toxicology & pharmacology : CBP*, 141(1), 50-7.

[113] Mortari, M. R., Cunha, A. O., de Oliveira, L., Vieira, E. B., Gelfuso, E. A., Coutinho-Netto, J., et al. (2005). Anticonvulsant and behavioural effects of the denatured venom of the social wasp Polybia occidentalis (Polistinae, Vespidae). *Basic & clinical pharmacology & toxicology*, 97(5), 289-95.

[114] Wang, C. G., He, X. L., Shao, F., Liu, W., Ling, M. H., Wang, D. C., et al. (2001). Molecular characterization of an anti-epilepsy peptide from the scorpion Buthus martensi Karsch. *Eur J Biochem*, 2268(8), 480-5.

[115] Zhao, R., Weng-C, C., Feng, Q., Chen, L., Zhang-Y, X., Zhu-Y, H., et al. (2011). Anticonvulsant activity of BmK AS, a sodium channel site 4-specific modulator. *Epilepsy & Behavior*, 20(2), 267-76.

[116] Martin, BM R. A., Gurrola, G. B., Nobile, M., Prestipino, G., Possani, L. D., & Novel, K. (1994). channel-blocking toxins from the venom of the scorpion Centruroides limpidus limpidusKarsch. *Biochem J*, 15(304), 51-6.

[117] Corona, M., Coronas, F. V., Merino, E., Becerril, B., Gutiérrez, R., Rebolledo-Antunez, S., et al. (2003). A novel class of peptide found in scorpion venom with neurodepressant effects in peripheral and central nervous system of the rat. *Biochimica et Biophysica Acta BBA)- Proteins & Proteomics*, 1649(1), 58-67.

[118] Cougle, J. R., Keough, Riccardi. C. J., & Sachs-Ericsson, N. (2009). Anxiety disorders and suicidality in the National Comorbidity Survey-Replication. *J Psychiatr Res*, 43(9), 825-9.

[119] Hatcher, S., & Arroll, B. (2012). Newer antidepressants for the treatment of depression in adults. *BMJ*, 344, d8300.

[120] Nemeroff, C. B., & Owens, M. J. (2002). Treatment of mood disorders. *Nat Neurosci. Suppl*, 5, 1068-70.

[121] Liberato, J. L., Cunha, A. O., Mortari, M. R., Gelfuso, E. A., Beleboni, Rde. O., Coutinho-Netto, J., et al. (2006). Anticonvulsant and anxiolytic activity of FrPbAII, a novel GABA uptake inhibitor isolated from the venom of the social spider Parawixia bistriata (Araneidae: Araneae). *Brain Res*, 1124(1), 19-27.

[122] Saidemberg, D., Ferreira, M., Takahashi, M. A., Gomes, T. N., Cesar-Tognoli, P. C., da, L. M., Silva-Filho, L. C., et al. (2009). Monoamine oxidase inhibitory activities of indolylalkaloid toxins from the venom of the colonial spider Parawixia bistriata: functional characterization of PwTX-I. *Toxicon*, 54(6), 717-24.

[123] Maruyama, M., & Na, W. Monoamine Oxidase Inhibitors as Neuroprotective Agents in Age-Dependent Neurodegenerative Disorders. *Curr Pharm Des*, 19, 799-817.

[124] Dwyer, J. M., Rizzo, S. J., Neal, S. J., Lin, Q., Jow, F., Arias, R. L., et al. (2009). Acid sensing ion channel (ASIC) inhibitors exhibit anxiolytic-like activity in preclinical pharmacological models. *Psychopharmacology (Berl)*, 203(1), 41-52.

[125] de Oliveira, L., Cunha, A. O., Mortari, M. R., Coimbra, N. C., & Dos, Santos. W. F. (2006). Cataleptic activity of the denatured venom of the social wasp Agelaia vicina (Hymenoptera, Vespidae) in Rattus norvegicus (Rodentia, Muridae). *Prog Neuropsychopharmacol B ol Psychiatry*, 30(2), 198-203.

[126] de Oliveira, L., Cunha, A. O., Mortari, M. R., Pizzo, A. B., Miranda, A., Coimbra, N. C., et al. (2005). Effects of microinjections of neurotoxin AvTx8, isolated from the social wasp Agelaia vicina (Hymenoptera, Vespidae) venom, on GABAergic nigrotectal pathways. *Brain Res*, 1031(1), 74-81.

[127] Gati, C. D., Mortari, M. R., & Schwartz, E. F. (2012). Towards therapeutic applications of arthropod venom k(+)-channel blockers in CNS neurologic diseases involving memory acquisition and storage. *Journal of toxicology*, 756358.

[128] Dilly, S., Lamy, C., Marrion, N. V., Liegeois, . J. F., & Seutin, . V. (2011). Ion-channel modulators: more diversity than previously thought. *Chembiochem : a European journal of chemical biology*, 12(12), 1808-12.

[129] Romero-Curiel, A., Lopez-Carpinteyro, D., Gamboa, C., De la Cruz, F., Zamudio, S., & Flores, G. (2011). Apamin induces plastic changes in hippocampal neurons in senile Sprague-Dawley rats. *Synapse*, 65(10), 1062-72.

[130] Gimenez-Gallego, G., Navia, MA, Reuben, J. P., Katz, G. M., Kaczorowski, G. J., & Garcia, M. L. (1988). Purification, sequence, and model structure of charybdotoxin, a

potent selective inhibitor of calcium-activated potassium channels. *Proc Natl Acad Sci U S A*, 85(10), 3329-33.

[131] Crest, M., Jacquet, G., Gola, M., Zerrouk, H., Benslimane, A., Rochat, H., et al. (1992). Kaliotoxin, a novel peptidyl inhibitor of neuronal BK-type Ca(2+)-activated K+ channels characterized from Androctonus mauretanicus mauretanicus venom. *J Biol Chem*, 267(3), 1640-7.

[132] Galvez, A., Gimenez-Gallego, G., Reuben, J. P., Roy-Contancin, L., Feigenbaum, P., Kaczorowski, G. J., et al. (1990). Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion Buthus tamulus. *J Biol Chem*, 265(19), 11083-90.

[133] Kourrich, S., Mourre, C., & Soumireu-Mourat, B. (2001). Kaliotoxin, a Kv1.1 and Kv1.3 channel blocker, improves associative learning in rats. *Behav Brain Res*, 120(1), 35-46.

[134] Edwards, T. M., & Rickard, N. S. (2006). Pharmaco-behavioural evidence indicating a complex role for ryanodine receptor calcium release channels in memory processing for a passive avoidance task. *Neurobiol Learn Mem*, 86(1), 1-8.