Toxicon 76 (2013) 328–342

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Review

Scorpion venom components that affect ion-channels function

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ARTICLE INFO

Article history:
Received 6 March 2013
Accepted 16 July 2013
Available online 26 July 2013

Keywords:
Biodiversity
Ion-channel
Functional effect
Scorpion toxin
Structural features

ABSTRACT

The number and types of venom components that affect ion-channel function are reviewed. These are the most important venom components responsible for human intoxication, deserving medical attention, often requiring the use of specific anti-venoms. Special emphasis is given to peptides that recognize Na^+-, K^+- and Ca^{++}-channels of excitable cells. Knowledge generated by direct isolation of peptides from venom and components deduced from cloned genes, whose amino acid sequences are deposited into databanks are nowadays in the order of 1.5 thousands, out of an estimate biodiversity closed to 300,000. Here the diversity of components is briefly reviewed with mention to specific references. Structural characteristic are discussed with examples taken from published work. The principal mechanisms of action of the three different types of peptides are also reviewed. Na^+-channel specific venom components usually are modifier of the open and closing kinetic mechanisms of the ion-channels, whereas peptides affecting K^+-channels are normally pore blocking agents. The Ryanodine Ca^{++}-channel specific peptides are known for causing sub-conducting stages of the channels conductance and some were shown to be able to internalize penetrating inside the muscle cells.

1. Introduction

Scorpion stings are a public health problem in certain countries, with an estimate over one million accidents in humans, annually (Chippaux and Goyffon, 2008), which certainly requires medical attention, due to high number of fatalities, which is approximately 2600 per year (Chippaux, 2012). Scorpions have been classified into 18 different families (Prendini and Wheeler, 2005) of which 30 genera belonging to the family Buthidae presents human treat, and they constitute circa 25% of the world biodiversity (possible in the order of 2000 different species). The most important species causing human accidents are scorpions of the genus: *Androctonus*, *Buthus*, and *Leiurus*, in North Africa and Middle East, *Centruroides* and *Tityus* in the American continent, *Mesobuthus* in Asia, *Parabuthus* in South Africa (Caliskan et al., 2013).

Scorpion venoms are a complex mixture of substances, among which are: inorganic salts, free amino acids, heterocyclic components, peptides and proteins, mainly enzymes, which are used by the scorpions for defense and capture of prays. Each scorpion has its own arsenal of components for these purposes. The known number of different components in their venoms varies from 72 (*Androctonus mauretanicus mauretanicus*) to over 600 (*Mesobuthus tumulus*, and *Tityus serrulatus*) (Oukkache et al., 2008; Newton et al., 2007; Batista et al., 2007). But the world biodiversity is in the order of 300,000, if we

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0041-0101/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxicon.2013.07.012

assume each scorpion having 150 different components, from which approximately 750 proteins are registered in The Animal Annotation Program of UniProt (www.uniprot.org/program/Toxins), and at least the double of this number is known after translating the known nucleotide sequences of genes cloned from scorpions. Yet, this is less than 1% of the total expected number of distinct components to exist in scorpion venoms. The best known components are peptides that modify ion-channel permeability of both excitable and non excitable cells, belonging to a single structural class built around a scaffold known as Cystine-Stabilized α/β (CS-α/β) motif. Most of the data mentioned above can easily be revised in the recent publication, dealing with the mining aspects of scorpion venom components (Rodríguez de la Vega et al., 2013). However, due to the increment of new strategies of proteome analysis and gene cloning from transcriptomes, the number of identified components has increased significantly. The new approaches have indicated that many other structural motifs and possible functions are being found and will be identified in the near future (Rodríguez de la Vega et al., 2013).

The General classification of scorpion toxins (Possani et al., 1999; Tytgat et al., 1999; Rodríguez de la Vega and Possani, 2004; Tan et al., 2006a) is based on four different criteria: the ion-channel involved (sodium, potassium, calcium and chlorine), the specific receptor to which the toxin binds to, the three-dimensional structure of the toxin and the type of response induced (activation/inactivation of the receptor).

In this review will focus our attention to the classical peptides, normally called toxins that modify the gating mechanism of Na^+-channels, block K^+-channel or modulate the function of calcium channel sensitive to Ryanodine.

2. Na^+-channel specific toxins

The venom of scorpions contains several types of toxins that may interact with each other, modulating the function of ion channels, usually being responsible for the known multiple symptoms of poisoning. On the medical viewpoint, the toxins that bind to sodium channels of mammals, particularly humans, are the most important ones. These toxins are polypeptides of 61–76 amino acid residues in length, tightly bound by four disulfide bridges (Possani et al., 1999) and currently are classified into two categories, based on their physiological effects on channel gating and their binding properties: alpha-toxins (α-NaScTxs), which bind at receptor site 3 on the extracellular surface of the channel and inhibit the fast inactivation process (Couraud et al., 1982; Meves et al., 1986 and reviewed in Bosmans and Tytgat, 2007), and beta-toxins (β-NaScTxs), which bind to receptor site 4 and shift the threshold of the channel activation to more negative membrane potentials (reviewed in Rodríguez de la Vega and Possani, 2007; Weinberger et al., 2010).

2.1. Scorpion α-NaScTxs

Scorpion α-NaScTxs were initially described for species collected in the "Old World" (Africa and Asia), but latter also described for scorpions of the "New World" (America) (reviewed in Gordon et al., 2003). The α-NaScTxs are subdivided into distinct groups (Catterall, 1992; Gordon et al., 1998 and reviewed in Gordon et al., 2007):

(1) Classical α-toxins, are highly active only in mammalian voltage-gated sodium channels (VGSCs) with high affinity (Kd in the range of 0.2–5 nM) to rat brain synaptosomes. Among these toxins are: Aah2, Aah1 and Aah3 from *Androctonus australis* Hector, Lqq5 from *Leiurus quinquestriatus quinquestriatus* and Bot3 from *Buthus occitanus tunetatus*, peptides purified from North African scorpions (Martin-Eauclaire and Couraud, 1995; Froy and Gurevitz, 2003). From the venom of scorpions of the 'New World' peptides isolated from *T. serrulatus* and *Centruroides sculpturatus* have also been classified as α-NaScTxs (Meves et al., 1984; Possani et al., 1999).

(2) Anti-insect α-NaScTXs, that are highly active only on insect VGSCs. Examples of these toxins are: LqhαIT (Eitan et al., 1990), Lqq3 (Kopeyan et al., 1993), and BotIT1 (Borchani et al., 1997), which bind with high affinity to insect neuronal preparations (0.06–1 nM).

(3) α-Like toxins, active on both insect and mammalian VGSCs. Examples are: Lqh3 and Lqh6 (from *L. quinquestriatus hebraeus*), Bom3 and Bom4 (from *B. occitanus mardochei*), and BmK M1 (from *Buthus martensii* Karsch).

2.2. Scorpion β-NaScTxs

β-NaScTxs are classified into four well supported phylogenetic branches or subclasses (reviewed in Pedraza Escalona and Possani, 2013): (1) anti-mammalian β-toxins exclusively found in scorpions of the genus *Centruroides*. Examples are Cn2 from *Centruroides noxius* and Css4 from *Centruroides suffusus suffusus* (Vazquez et al., 1995; Martin et al., 1987); (2) β-toxins active on both insect and mammalian VGSCs, such as Ts1 from *T. serrulatus* (Possani et al., 1985) and Lqhβ1 from *L. quinquestriatus hebraeus* (Gordon et al., 2003); (3) anti-insect selective excitatory β-toxins such as AahIT from *Androctonus australis* Hector and Bj-xtrIT from *Hotentota judaica*, that causes contraction paralysis in fly larvae (Pélate and Zlotkin, 1982; Zlotkin et al., 1985; Froy et al., 1999); and finally (4) anti-insect selective depressant toxins, which induce flaccid paralysis upon injection. Example is peptide LqhIT2 from *L. quinquestriatus hebraeus* (Zuo and Ji, 2004; Gurevitz et al., 2007).

2.3. Amino acid sequences of NA^+-channels toxins

The first scorpion toxins sequenced were purified from North African scorpions of the genus *Androctonus* (Rochat et al., 1967), but also from American scorpions (Babin et al., 1975). The work was performed after chromatographic separation of components of venoms and the