Toxicon 115 (2016) 63–69

Contents lists available at ScienceDirect

Toxicon

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

Venom from the spider *Araneus ventricosus* is lethal to insects but inactive in vertebrates

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ARTICLE INFO

Article history:
Received 3 December 2015
Received in revised form
26 February 2016
Accepted 15 March 2016
Available online 16 March 2016

Keywords:
Araneus ventricousus
Venom composition
Electrophysiological analysis
Voltage-gated sodium channels (VGSCs)

ABSTRACT

Araneus ventricosus spider venom, which was collected by electrical stimulation, is abundant in peptides and proteins with molecular weights ranging from 2 kDa to 70 kDa as determined by gel electrophoresis and mass spectrometry. Electrophysiological experiments showed that 50 μg/mL venom could block the voltage-gated sodium channels (VGSCs) currents of the dorsal unpaired median (DUM) neurons of *Periplaneta americana* cockroaches. However, 500 μg/mL venom could not block the VGSCs currents in rat dorsal root ganglion cells or the neuromuscular transmission in isolated mouse phrenic nerve-hemidiaphragm. Moreover, we also observed that injection of the venom in *P. americana* gave rise to obvious envenomation symptoms, with a LD_{50} value of 30.7 μg/g. Enzymatic analysis indicated that the venom possessed activities of several kinds of hydrolases including hyaluronidase and proteases. These results demonstrate that *A. ventricosus* venom contains bioactive components targeting insects, which are the natural prey of these spiders. Furthermore, the venom was found to be not active in vertebrate. Thus, we suggest that *A. ventricosus* venom contains novel insect-selective compounds that might be helpful in developing new and safe insecticides.

1. Introduction

In order to capture prey or defend themselves, spiders have developed potent neurotoxins and other biologically active compounds in their venom. Spider venoms are complex mixtures of biologically active compounds, from salts to large multidomain proteins (Schanbacher et al., 1973; Ori and Ikeda, 1998; Vassilevski et al., 2009; Kuhn-Nentwig et al., 2011). The extraordinary chemical and pharmacological complexity of spider venoms has encouraged many scientists to systematically analyze these venoms in an attempt to unveil candidates for drug and pesticide discovery (Windley et al., 2011, 2012), and as well as tools to study important receptors and ion channels (King, 2011). With spiders being the most successful insect predators on this planet, it seems logical that the majority of spider venom compounds are insecticidal. As far as the pharmacology and biochemistry of spider venom is concerned, spider venom contains a wide variety of ion channel toxins, novel non-neurotoxins, enzymes and low molecular weight compounds (Adams, 2004; Ushkaryov et al., 2004; Kuhn-Nentwig et al., 2004; Escoubas et al., 2000 & Escoubas and rash, 2004; Liang, 2004; Li et al., 2005; Duan et al., 2008; King and Hardy, 2013).

Up to the present, there are 45,841 described spiders (World Spider Catalog, 2016), with an even greater number awaiting characterization (Coddington and Levi, 1991). Despite this diversity, peptide toxins have so far been studied from the venom of only 97

Abbreviations: DRG, dorsal root ganglion; DUM neurons, dorsal unpaired median neurons; HEPES, N-hydroxyethyl piperazine-N-ethanesulfonic acid; LD50, median lethal dose; MALDI-TOF, matrix assisted laser desorption/ionization-time of flight; RP-HPLC, reverse phase – high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; VGSCs, voltage-gated sodium channels.

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http://dx.doi.org/10.1016/j.toxicon.2016.03.010
0041-0101/

spider species (Herzig et al., 2011). Within the genus *Araneus*, only the venoms of few species have been studied, such as *Araneus gemma* and *Araneus diadematus*, which were reported to target glutamate receptors from vertebrates, insects or crustaceans (Usherwood et al., 1984; Michaelis et al., 1984; Vyklický et al., 1986). The nocturnal spider *Araneus ventricosus* belonging to genus *Araneus* are found in Russia, China, Korea, Taiwan, and Japan (World Spider Catalog, 2016). Spiders of the genus *Araneus* mainly feed on flies, mosquitos, and other invertebrates. Their venom is a complex mixture of components with diverse biological actions that can rapidly paralyze and kill insects. Although some studies have described bioactivies of venom from *A. ventricosus* antagonizing the glutamate receptor and killing the silkworm larvae (Kawai et al., 1983; Chung et al., 2002), a systematic physiological and biochemical analysis of the venom has not been available.

In the present study, we have therefore examined the composition and activity of *A. ventricosus* (Fig. 1) venom in more detail, using chromatographic as well as electrophysiological techniques. Furthermore, the enzymatic activity of the venom was examined and its bioactivity determined by injection into cockroaches and mice. Electrophysiological analysis revealed that *A. ventricosus* venom showed insecticidal activity due to venom components targeting voltage-gated sodium channels (VGSCs) in cockroach dorsal unpaired median (DUM) neurons, which has been well acknowledged as a useful tool for the identification of novel insecticides (Wang et al., 2012; Grolleau and Lapied, 2000; King, 2011; King and Hardy, 2013). Furthermore, we did neither observe any envenomation symptoms when injecting this venom into mice, nor any activity on rat dorsal root ganglion (DRG) neurons or in the mouse phrenic nerve-hemidiaphragm preparation. It can therefore be concluded that *A. ventricosus* venom contains insecticidal compounds, which seem to be devoid of any vertebrate activity.

2. Materials and methods

2.1. Chemicals

Trifluoroacetic acid (TFA), dithiothreitol (DTT), iodoacetamide (IAA), acrylamide, N, N′-methylene bisacrylamide (Bis), Glycine, trihydroxymethyl aminomethane (Tris), sodium dodecyl sulfate (SDS) and SDS-PAGE protein standards were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Bromophenol blue, N, N, N′, N′-tetramethylethylenediamine (TEMED) were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Phenylmethylsulfonyl fluoride (PMSF) was from Ameresco. HPLC-grade acetonitrile (ACN) was purchased from Hunan Fine Chemistry Institute (Hunan, China). Casein, cetyltrimethylammonium bromide, hyaluronate were from Sangon biotech Co., Ltd. Acetylcholinesterase, Alkaline phosphatase and Acid phosphatase kit were from Nanjing jiancheng bioengineering institute (China).

Fig. 1. *Araneus ventricosus*.

2.2. Venom collection

Spiders captured in the wild were used for venom extraction. The venom was collected from female spider *A. ventricosus* using a method described in our previous study (Wang et al., 2007), then the venom was freeze-dried and stored at −80 °C before analysis.

2.3. Enzyme activity and protein analysis

The determination of hydrolase hyaluronidase (Ferrante, 1956), proteinase (Rich, 1963), alkaline phosphatase (Bessey et al., 1946), acid phosphatase (Bessey et al., 1946) and acetylcholine esterase (Pilz, 1963) were performed according to the methods described previously. Protein content of the venom was determined using Bradford method (Bradford, 1976). SDS-PAGE of the venom was performed according to the method of Laemmli (Laemmli, 1970) under denatured conditions in a 14% polyacrylamide slab gel. Venom sample (12 μg) was denatured and reduced in SDS loading buffer and boiled for 3 min. The venom solution was centrifuged for 10 min at room temperature (25 °C) and 10,000 g, then the supernatant was loaded into the gel well. SDS-PAGE was run at 25 mA on polyacrylamide stacking gel and at 50 mA on separating gel. After completion of electrophoresis, the proteins in gel were visualized by Coomassie Brilliant Blue G-250. The low molecular weight calibration Kit (Fermentas, USA) was used as standard molecular weight marker proteins.

2.4. Toxin purification with RP-HPLC

The crude venom was analyzed on a reversed-phase C4 column (4.6 mm × 250 mm, Sepax Technologies, USA) using a Waters HPLC Alliance system (Waters, USA) with a 996 photodiode array detector. Mobile phase A was 0.1% aqueous TFA and mobile phase B was acetonitrile containing 0.1% TFA. After the sample was loaded, components were eluted for 60 min with the following gradient of solvent B: 0–22 min, 0–20%; 22–26 min, 20–40%; 26–56 min, 40–80%. Flow rate was maintained at 0.7 mL/min, and effluent absorbance was recorded at 280 nm. Eluates were respectively collected and lyophilized, stored at −20 °C.

2.5. Mass spectrometric analysis

Mass spectrometry was used to detect the peptides or proteins with molecular weight below 20 kDa. The analysis was performed in AB Sciex-TOF/TOF 5800 mass spectrometer (Applied Biosystems, USA) by choosing the matrixα-Cyano-4-hydroxycinnamic acid (CHCA) (Sigma–Aldrich, USA), 0.5 μL (10 mg/mL) mixed with 0.5 μL venom sample (20 μg/mL). Mass spectra were recorded under the control of TOF/TOF series explorer software (Applied Biosystems, USA). MALDI-TOF spectra were recorded in the positive ion linear mode over a mass range of 1000–20,000 Da. Linear final detector voltage was 1.92 kV. Laser intensity was 3800. An external mass calibration was done daily.