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Characterization of ion channels on subesophageal ganglion neurons from Chinese tarantula *Ornithoctonus huwena*: Exploring the myth of the spider insensitive to its venom

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ABSTRACT

Chinese tarantula *Ornithoctonus huwena* is one of the most venomous spiders distributing in the hilly areas of southern China. In this study, using whole-cell patch-clamp technique we investigated electrophysiological and pharmacological properties of ion channels from tarantula subesophageal ganglion neurons. It was found that the neurons express multiple kinds of ion channels at least including voltage-gated calcium channels, TTX-sensitive sodium channels and two types of potassium channels. They exhibit pharmacological properties similar to mammalian subtypes. Spider calcium channels were sensitive to ω-conotoxin GVIA and diltiazem, two well-known inhibitors of mammalian neuronal high-voltage-activated (HVA) subtypes. 4-Aminopyridine and tetraethylammonium could inhibit spider outward transient and delayed-rectifier potassium channels, respectively. Huwentoxin-I and huwentoxin-IV are two abundant toxic components in the venom of *Ornithoctonus huwena*. Interestingly, although in our previous work they inhibit HVA calcium channels and TTX-sensitive sodium channels from mammalian sensory neurons, respectively, they fail to affect the subtypes from spider neurons. Moreover, the crude venom has no effect on delayed-rectifier potassium channels and only slightly reduces transient outward potassium channels with an IC_{50} value of ~51.3 mg/L. Therefore, our findings provide important evidence for ion channels from spiders having an evolution as self-defense and prey mechanism.

1. Introduction

During about 400 million years of evolution from an arachnid ancestor, spiders have become a diverse group and successful terrestrial invertebrates. There are at least 40,000 described species in the world (Hormiga and Griswold, 2014; Rash and Hodgson, 2002). Spider venoms contain a large number of biologically active substances that selectively target a variety of vital physiological functions in both insects and mammals. Some of them can target voltage-gated ion channels and therefore disturb the electrophysiological properties of excitable cells (Catterall et al., 2007; Tao et al., 2013).

Chinese tarantula *Ornithoctonus huwena* (*O. huwena*) is one of the most venomous spiders distributing in the hilly areas of

Abbreviations: HWTX, Huwentoxin; ICK, inhibitor cystine knot; DRG, dorsal root ganglion; TTX, tetrodotoxin; TTX-S, TTX-sensitive; IC_{50}, median inhibitory concentration; HPLC, high pressure liquid chromatography.

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southern China. The venom of this spider contains a mixture of compounds with different types of biological activity (Liang, 2004). For example, Huwentoxin-I (HWTX-I) is a 33-residue polypeptide with three disulfide bonds. It inhibits both mammalian N-type calcium channels and neuronal TTX-sensitive (TTX-S) sodium channels (Wang et al., 2007). Huwentoxin-IV (HWTX-IV) is a 35-residue polypeptide with three disulfide bridges. Like HWTX-I, HWTX-IV inhibits neuronal TTX-S sodium channels from adult rat dorsal root ganglion (DRG) neurons. More recently, HWTX-IV is found to preferentially inhibit neuronal sodium channel subtypes such as Nav1.2, Nav1.3 and Nav1.7 compared to the muscle subtypes Nav1.4 and Nav1.5 (Deng et al., 2013; Liu et al., 2013). Huwentoxin-V (HWTX-V), composing of 35 amino acid residues, is an insect neurotoxic peptide and can specifically inhibited high-voltage-activated (HVA) calcium channels from adult cockroach dorsal unpaired median (DUM) neurons (Deng et al., 2008; Zhang et al., 2003). Three-dimensional structures of HWTX-I and HWTX-IV are also determined by ^{1}H 2D NMR method (Peng et al., 2002; Qu et al., 1997), revealing that they belong to the family of inhibitory cysteine knot (ICK) peptides frequently emerging in spiders and marine cone snails.

These toxins are powerful weapons for spiders to aid the capture of prey or defense against predators. Theoretically, to the benefit of self-defense, spiders might evolve ion channels that are resistant to these toxins. Scorpion is another arachnid that secrets peptide toxins. Legros et al. demonstrated that its own venom is pharmacologically inactive on potassium channels and sodium channels from the scorpion (Legros et al., 1998). However, electrophysiological and pharmacological properties of spider ion channels remain unknown. In this study, using whole-cell recording technique we found that at least three kinds of ion channels express on subesophageal ganglion neurons from tarantula *O. huwena*, including: (i) HVA calcium channels, (ii) outward transient and delayed-rectifier potassium channels and (iii) TTX-S sodium channels. Interestingly, three major toxins (HWTX-I, HWTX-IV, HWTX-V) in *O. huwena* venoms exhibit much lower affinities for spider ion channels than for mammalian isoforms.

2. Materials and methods

2.1. Venom collection and toxin purification

Experiments were carried out with Chinese tarantula *O. huwena*. Spiders were collected from the hilly area of Ninming county of Guanxi Province in the south of China. Spider venom was obtained by electrical stimulation of female *O. huwena* as described in our previous work (Tao et al., 2016). The freeze-dried crude venom was stored at −20 °C prior to analysis. HWTX-I, HWTX-IV and HWTX-V were purified by a combination of ion-exchange and reverse-phase high-performance liquid chromatography (RP-HPLC) as described earlier (Deng et al., 2014).

2.2. Cell isolation procedures

Spider subesophageal ganglion neurons were acutely dissociated and maintained in a short-term primary culture according to the procedures below. Briefly, female spiders (*O. huwena*) were killed in 75% alcohol and washed in distilled water. After wiping off the sternum, the subesophageal ganglion was excised, and then transferred into saline solution (in mM): 200 NaCl, 3 KCl, 5 $CaCl_2$, 4 $MgCl_2$, 50 sucrose and 10 HEPES at pH 7.4. The isolated subesophageal ganglion neurons were suspended in culture medium containing (in mM): 223 NaCl, 6.8 KC1, 8 $CaCl_2$, 5 $MgCl_2$, 5 Sucrose, 10 HEPES, 1 Glutamine, 20% Bovin Calf Serum, penicillin and streptomycin at pH 7.4. The cells were incubated at 28 °C for 2–3 h before patch clamp experiment.

Rat dorsal root ganglion (DRG) neurons were acutely dissociated and maintained in a short-term primary culture according to the procedures adapted from Deng et al. (Deng et al., 2014). Briefly, 30-day adult Sprague-Dawley rats of either sex were killed under deep ethyl ether anaesthesia followed by decapitation, the dorsal root ganglia were removed quickly from the spinal cord, and then transferred into Dulbecco's modified eagle's medium (DMEM) containing trypsin (0.5 g/l, type III), collagenase (1.0 g/l, type IA) to incubate at 34 °C for 30 min. Enzyme treatment was terminated with trypsin inhibitor (1.5 g/l, type II-S). The isolated DRG cells were suspended in essential DMEM medium supplemented with 10% newborn calf serum and $NaHCO_3$ (3.6 g/l). Finally, the cells were plated in 35-mm culture dishes (Corning, Sigma) and incubated in CO_2 incubator (5% CO_2, 37 °C). Cells were incubated for 3–24 h before patch clamp experiment.

2.3. Whole-cell patch clamp experiments

Macroscopic currents were recorded using an EPC-9 patch-clamp amplifier (HEKA Electronics, Germany) under whole-cell patch clamp configuration at room temperature (22–25 °C). Micropipettes (2–3 μm diameters) were pulled from borosilicate glass capillary tubing by using a two-step vertical puller (PC-10, Narishige, Olypmus). The resistances of recording pipettes were 1.0–2.0 MΩ when filled with internal solution. The currents were monitored for 10 min; the cells showing noticeable run-down currents during this period were discarded. Series resistance was kept near 5 MΩ and compensated by 65–70%. Linear capacitance and leakage currents were digitally subtracted using P/4 protocol.

Calcium channel currents were measured using Ba^{2+} as a charge carrier. Calcium currents were recorded using internal solution containing (in mM): 140 CsCl, 2 $MgC1_2$, 2 ATP-Mg, 10 HEPES and 1.5 EGTA at pH 7.2, and bathing solution containing (in mM): 150 Choline Chloride, 2 $MgC1_2$, 10 $BaC1_2$, 10 HEPES, 25 TEA and 1 tetrodotoxin at pH 7.4.

For potassium currents recording, bathing solution contained (in mM): 130 choline chloride, 5 KOH, 10 HEPES, 12 glucose, 2 $MgCl_2$, 2 $CaCl_2$ at pH 7.2. Internal solution contained (in mM): 120 KF, 20 NMG (N-methyl-D-glucamine), 10 HEPES, 11 EGTA, 2 MgATP, 0.5 Li_2GTP at pH 7.2.

Sodium currents were recorded using internal solution containing (mM): CsF 135, NaCl 10, Hepes 5 at pH 7.0 and bathing solution containing (mM): NaCl 30, CsCl 5, D-glucose 25, MgCl2 1, CaCl2 1.8, HEPES 5, tetraethylammonium chloride 20, tetramethylammonium chloride 70 at pH 7.4.

2.4. Data analysis

Experimental data were acquired and analyzed by the program Pulse-Pulsefit8.0 (HEKA, Germany) and Sigmaplot (Sigma, USA). All data are presented as mean ± standard error and *n* is the number of independent experiments. Dose–response curves to determine IC_{50} values were fitted using the following form of the Hill equation (1): $y = 1/[1 + ([x]/Dose_{50})^{H}]$, where *x* is the toxin dose, *H* is the Hill coefficient (slope parameter), and $Dose_{50}$ is the median inhibitory dose causing lethality or block of membrane currents, respectively.

On-rates were determined by fitting time course data with the following single exponential decay function (2): $y = Ae^{-kx} + C$, where *x* is the time, *A* is the normalized current value (usually1.0) before application of toxin, and *C* is the final normalized current value following block by the toxin. The on-rate (τ_{on}) was determined from the inverse of the rate constant *k*.