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Synthesis and characterization of huwentoxin-IV, a neurotoxin inhibiting central neuronal sodium channels ☆

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Abstract

Our previous work demonstrated that huwentoxin-IV was an inhibitor cystine knot peptide from Chinese tarantula *Ornithoctonus huwena* venom that blocked tetrodotoxin-sensitive voltage-gated sodium channels from mammalian sensory neurons [Peng, K., Shu, Q., Liu, Z., Liang, S., 2002. Function and solution structure of huwentoxin-IV, a potent neuronal tetrodotoxin (TTX)-sensitive sodium channel antagonist from Chinese bird spider *Selenocosmia huwena*. J. Biol. Chem. 277(49), 47564–47571]. However, the actions of the neurotoxin on central neuronal sodium channels remain unknown. In this study, we chemically synthesized native huwentoxin-IV and found that sodium channel isoforms from rat hippocampus neurons were also sensitive to native and synthetic toxins, but the toxin-binding affinity (IC_{50}~0.4 μM) was 12-fold lower than to peripheral isoforms. The blockade by huwentoxin-IV could be reversed by strong depolarization due to the dissociation of toxin–channel complex as observed for receptor site 3 toxins. Moreover, small unilamellar vesicle-binding assays showed that in contrast to ProTx-II from the tarantula *Thrixopelma pruriens*, huwentoxin-IV almost lacked the ability to partition into the negatively charged and neutral phospholipid bilayer of artificial membranes. These findings indicated that huwentoxin-IV was a sodium channel antagonist preferentially targeting peripheral isoforms via a mechanism quite different from ProTx-II.

Keywords: Spider; Toxin; Sodium channel; Whole-cell recording; Hippocampus

1. Introduction

The opening of voltage-gated sodium channels is responsible for the rapid rising phase and propagation of action potentials, and they therefore play a critical role in the specialized cell functions such as excitability, contraction, secretion and synaptic transmission (Ren et al., 2001). To date, at least nine α subunit isoforms ($Na_v1.1$–1.9) have been cloned and characterized from mammals. They have special developmental, tissue or cellular distributions (Cummins et al., 2001). Although multiple isoforms can be detected in sensory neurons, $Na_v1.6$–1.9 channels are preferentially expressed in dorsal root ganglia neurons whereas $Na_v1.1$–1.3 channels are dominant in central neurons (Ogata

☆ *Ethical statement:* In this study, our mission is to disclose the natural properties of the peptide toxin from spider venom. The procedure that we deal with all animals is approved by the Animal/Human Research Ethics Committee of Hunan Normal University.

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and Ohishi, 2002). Importantly, there is considerable evidence demonstrating that certain sodium channel subtypes such as $Na_v1.7$, 1.8 and 1.9 are involved in pain sensation (Benarroch, 2007; Villarreal et al., 2005; Black et al., 2004; Nassar et al., 2004; Cox et al., 2006). Nine sodium channel subtypes show over 75% sequence identity to each other. Ligands selectively targeting sodium channel subtypes are suggested to be important tools for investigating structure–functional relationships of sodium channels and to be potential candidates for developing novel analgesics.

Many peptide toxins from the venoms of scorpion, cone snail and spider are found to interact with peripheral neuronal sodium channels. They can modulate the activities of sodium channels through different mechanisms to affect neuronal excitability. Most of them modulate channel activation or inhibit inactivation by binding to receptor sites 4 (scorpion β-toxin) and 3 (scorpion α-toxins and spider δ-toxins), respectively, whereas μ-conotoxins binding to receptor site 1 block channel pore (see reviews: Bosmans and Tytgat, 2007; Nicholson et al., 2004; Li and Tomaselli, 2004). These toxins may target several sodium channel isoforms, but they show distinct affinity to these isoforms. For example, $Na_v1.7$ is 250-fold more sensitive to OD1, a toxin from the scorpion *Odonthobuthus doriae*, than $Na_v1.3$ (Maertens et al., 2006).

Huwentoxin-IV (HWTX-IV) is a 35-residue polypeptide from the tarantula *Ornithoctonus huwena* venom, and its structure represents a typical inhibitor cystine knot motif frequently emerging in spiders and cone snails (Peng et al., 2002). Similarly to hainantoxin-III and -IV from the tarantula *Ornithoctonus hainana* (Xiao and Liang, 2003), HWTX-IV specifically blocks TTX-sensitive sodium currents on rat DRG neurons, while having no effect on TTX-resistant isoforms. We also obtain the cDNA sequence of the toxin that encodes a precursor with 87 residues (Diao et al., 2003). In this study, we chemically synthesized the peptide toxin and examined the properties of the neurotoxin on central sodium channels from rat hippocampus neurons. Our data demonstrated that HWTX-IV was synthesized successfully and was a neurotoxin preferentially inhibiting peripheral neuronal sodium channels. The inhibition of sodium currents could be reversible by strong depolarization due to the dissociation of toxin channels complex. In contrast to ProTx-II, a sodium channel toxin from the venom of the tarantula *Thrixopelma pruriens* (Smith et al., 2005; Priest et al., 2007), HWTX-IV lacked the ability to partition into phospholipid bilayer of artificial membranes.

2. Materials and methods

2.1. Toxin purification

HWTX-IV was purified from Chinese tarantula *O. huwena* venom as previously described (Peng et al., 2002).

2.2. Peptide synthesis, folding and purification

The synthesis of native HWTX-IV was carried out on an automatic peptide synthesizer (PerSeptive Biosystems) using an Fmoc/*tert*-butyl strategy and HOBt/TBTU/NMM coupling method. The first C-terminal residue (I) was coupled to a poly(ethylene glycol) polystyrene resin that was equipped with a peptide amide linker (Fmocpeptide amide linker–polyethylene glycol–polystyrene (PAL-PEG-PS) amide resin, Applied Biosystems). After completion of synthesis, the peptide was cleaved from the resin with simultaneous removal of side chain protective groups by treatment with reagent K (82.5% trifluoroacetic acid, 5% double distilled H_2O, 5% phenol, 5% thioanisole and 2.5% ethanedithiol) for 2 h at room temperature (Li et al., 2004). Cold ether was added to the acidic mixture to give a precipitate at 4 °C. After washing twice with cold ether, the collected precipitate was lyophilized at −40 °C. The crude reduced peptides were purified by semipreparative RP-HPLC using a 50-min linear gradient of 10–45% solution B (0.1% trifluoroacetic acid in acetonitrile) in solution A (0.1% trifluoroacetic acid in double distilled H_2O) over 60 min on a C18 column at a 3 ml/min flow rate. When the purity of the peptide of interest was determined to be more than 95% by MALDI-TOF mass spectrometry and HPLC techniques, the peak was pooled and lyophilized. The linear-reduced peptide was dissolved in 0.1 M Tris–HCl buffer (pH 8.0) at a final concentration of 30 μM glutathione containing 5 mM reduced glutathione and 0.5 mM oxidized glutathione. The oxidization and folding was performed at room temperature and monitored at 280 nm by analytical RP-HPLC and MALDI-TOF mass spectrometry. The gradient of acetonitrile was as follows: from 0% to 10% of