ELSEVIER

Toxicon 48 (2006) 211–220

TOXICON

www.elsevier.com/locate/toxicon

A new toxin from the sea anemone *Condylactis gigantea* with effect on sodium channel inactivation

Ludger Ständker[a,*], László Béress[a,b], Anoland Garateix[c], Torsten Christ[d], Ursula Ravens[d], Emilio Salceda[e], Enrique Soto[e], Harald John[a], Wolf-Georg Forssmann[a], Abel Aneiros[c,✠]

[a]*IPF PharmaCeuticals GmbH, Feodor-Lynen Straße 31, 30625 Hannover, Germany*
[b]*Institut für Experimentelle Toxikologie, Universitäts-Klinikum Schleswig-Holstein, Brunswicker Street 10, 24105 Kiel, Germany*
[c]*Centre of Marine Bioproducts (CEBIMAR), Loma y 37, Vedado, Ciudad Habana, Cuba*
[d]*Institut für Pharmakologie und Toxikologie, Medizinischen Fakultät der TU Dresden, Fetscherstraße 74, 01307 Dresden, Germany*
[e]*Institute of Physiology, University Autonomous of Puebla, Mexico, 14 Sur 6301, CP 72570, Puebla, México*

Received 16 January 2006; received in revised form 4 May 2006; accepted 8 May 2006
Available online 19 May 2006

Abstract

A new peptide toxin exhibiting a molecular weight of 5043 Da (av.) and comprising 47 amino acid residues was isolated from the sea anemone *Condylactis gigantea*. Purification of the peptide was achieved by a multistep chromatographic procedure monitoring its strong paralytic activity on crustacea (LD_{50} approx. 1 μg/kg). Complete sequence analysis of the toxic peptide revealed the isolation of a new member of type I sea anemone sodium channel toxins containing the typical pattern of the six cysteine residues. From 11 kg of wet starting material, approximately 1 g of the peptide toxin was isolated. The physiological action of the new toxin from *C. gigantea* CgNa was investigated on sodium currents of rat dorsal root ganglion neurons in culture using whole-cell patch clamp technique ($n = 60$). Under current clamp condition (CgNa) increased action potential duration. This effect is due to slowing down of the TTX-S sodium current inactivation, without modifying the activation process. CgNa prolonged the cardiac action potential duration and enhanced contractile force albeit at 100-fold higher concentrations than the *Anemonia sulcata* toxin ATXII. The action on sodium channel inactivation and on cardiac excitation-contraction coupling resemble previous results with compounds obtained from this and other sea anemones [Shapiro, B.I., 1968. Purification of a toxin from tentacles of the anemone *C. gigantea*. Toxicon 5, 253–259; Pelhate, M., Zlotkin, E., 1982. Actions of insect toxin and other toxins derived from the venom of scorpion *Androtonus australis* on isolated giant axons of the cockroach *Periplaneta americana*. J. Exp. Biol. 97, 67–77; Salgado, V., Kem, W., 1992. Actions of three structurally distinct sea anemone toxins on crustacean and insect sodium channels. Toxicon 30, 1365–1381; Bruhn, T., Schaller, C., Schulze, C., Sanchez-Rodriquez, J., Dannmeier, C., Ravens, U., Heubach, J.F., Eckhardt, K., Schmidtmayer, J., Schmidt, H., Aneiros, A., Wachter, E., Béress, L., 2001. Isolation and characterization of 5 neurotoxic and cardiotoxic polypeptides from the sea anemone *Anthopleura elegantissima*. Toxicon, 39, 693–702]. Comprehensive analysis of the purified active fractions suggests that CgNa may represent the main peptide toxin of this sea anemone species.

Keywords: Peptide toxin; Na^+-channel inactivation; Positive inotropic effect; *Condylactis gigantea*; Sea anemone; Peptide purification

*Corresponding author. Tel.: +49 511 5466 329; fax: +49 511 5466 132.
E-mail address: L.standker@gmx.de (L. Ständker).
✠In memoriam.

0041-0101/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2006.05.001

1. Introduction

Sea anemones are sessile coelenterates living on solid cliffs on the floor of the ocean. They have large tentacles that incorporate and eliminate substances for metabolism and that contain venom-producing nematocysts allowing them to prey on small crustaceans and defend themselves against predators. These tentacles are well-known sources for the isolation of toxic proteins including pore-forming cytolisins (Wang et al., 2000; Anderluh and Macek, 2002), phospholipases (Grotendorst and Hessinger, 1999), Na^+-channel toxins (Norton, 1991; Béress, 2004), K^+-channel inhibitors (Kem, 1988; Aneiros et al., 1993; Castañeda et al., 1995; Schweitz et al., 1995; Diochot et al., 1998), and even proteinase inhibitors (Fritz et al., 1972; Antuch et al., 1993, Delfin et al., 1994). Several of the Na^+-channel toxins exhibit a high paralytic activity against crustacea.

Condylactis gigantea is, among others, a large sea anemone found abundantly in the Bermuda region and in the Caribbean. From *C. gigantea* the first partially characterized polypeptide toxin was isolated consisting of a crab-paralyzing basic polypeptide fraction with a molecular weight of 10–15 kDa (Shapiro, 1968; Shapiro and Lilleheil, 1969). Later this polypeptide toxin was described to interact with nerve membrane ionic conductances (Narahashi et al., 1969) and with sodium conductance of crayfish giant axon membranes (Murayama et al., 1972). Subsequent attempts to reproduce the original toxin-isolation procedure (Yost and O'Brian, 1978) yielded in the isolation of different peptide toxins in the molecular range of 6 kDa, but their exact biochemical properties are still unknown. Salgado and Kem (1992) reported the isolation of a new Na^+-channel toxin isolated from *C. gigantea*; however details about its molecular properties remain undescribed. Besides the well-characterized sea anemone toxins from *Anemonia sulcata*, *Anthopleura xanthogrammica* and *Anthopleura elegantissima* (reviewed by Norton, 1991; Béress, 2004), to date the molecular description of the main peptide toxins from *C. gigantea* remains unclarified. Here we describe the purification and present the complete primary structure of the main crab paralytic activity from *C. gigantea*. Its biological action on tetrodotoxin-sensitive sodium currents (TTX-S) of peripheral sensory neurons and on action potentials and force of contraction in mammalian myocardium is demonstrated.

2. Materials and methods

2.1. Toxin extraction and isolation

C. gigantea was collected from the Carribean sea close to Havana. The entire sea anemone body was washed with sea water to free them from attached sand and mud, immediately cooled to 0 °C, and later frozen at −20 °C. For peptide extraction, 11 kg of the sea anemone were mixed with 11 L of ethanol and 110 mL of glacial acetic acid was added. The mixture was homogenized portionwise in a Waring blendor, heated to 65 °C for denaturation of enzymes and structural proteins. After centrifugation at room temperature (3000 rpm), the supernatant was collected, and the remaining pellet was re-homogenized and re-extracted with 11 L of ethanol. The supernatants obtained were combined and a protein precipitation was started by adding 15 L ethanol to the 22-L anemone extract and left overnight at room temperature. The solution was filtered on a Seitz K 1000 filter and the filtrate was concentrated at reduced pressure (Rotavapor, Büchi, Essen, Germany) to a final volume of 2 L. A 10-fold amount of acetone was added to the concentrate by stirring and the mixture was left overnight at −20 °C in order to complete the precipitation of proteins and peptides. After 12 h the acetone supernatant was discarded, and the precipitate was re-dissolved in 2.5 L distilled water, filtered, and centrifuged at 5000 rpm.

Step 1 of purification: The resulting peptide-containing solution was applied to a Serdolit AD-2 column (5 × 50 cm i.d., Serva, Heidelberg, Germany) at a flow rate of 10 mL/min. The column was washed with 2.7 L one molar ammonium acetate, pH 5.4 and afterwards with 2.7 L water. Gradient elution was carried out using subsequently 2.1 L 1.2% (v/v) acetic acid/water; 3 L of 12.5% (v/v) acetic acid/water; and 4.7 L gradient volume from 12.5% to 50% (v/v) acetic acid/water; stepwise elution was carried out by 1.5 L 50% (v/v) acetic acid/water, 1.8 L 80% (v/v) acetic acid/water, and finally using 1.8 L 100% (v/v) acetic acid. Crab paralyzing activity was tested in all fractions.

All fractions were concentrated at reduced pressure (Rotavapur, Büchi) to 1/30 of their original volume.

Step 2 of purification: A 50 mL concentrate of the toxin fraction No. 9 was applied to a Sephadex G50 column (7 × 110 cm i.d, Pharmacia, Uppsala, Sweden) with a flow rate of 5 mL/min. The toxin