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AaTX1, from *Androctonus australis* scorpion venom: Purification, synthesis and characterization in dopaminergic neurons

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ABSTRACT

We have purified the AaTX1 peptide from the *Androctonus australis* (Aa) scorpion venom, previously cloned and sequenced by Legros and collaborators in a venom gland cDNA library from Aa scorpion. AaTX1 belongs to the α-Ktx15 scorpion toxins family (αKTx15-4). Characterized members of this family share high sequence similarity and were found to block preferentially I_A-type voltage-dependent K^+ currents in rat cerebellum granular cells in an irreversible way. In the current work, we studied the effects of native AaTX1 (nAaTX1) using whole-cell patch-clamp recordings of I_A current in substantia nigra pars compacta dopaminergic neurons. At 250 nM, AaTX1 induces 90% decrease in I_A current amplitude. Its activity was found to be comparable to that of rAmmTX3 (αKTx15-3), which differs by only one conserved (R/K) amino acid in the 19th position suggesting that the difference between R19 and K19 in AaTX1 and AmmTX3, respectively, may not be critical for the toxins' effects. Molecular docking of both toxins with Kv4.3 channel is in agreement with experimental data and suggests the implication of the functional dyade K27-Y36 in toxin-channel interactions. Since AaTX1 is not highly abundant in Aa venom, it was synthesized as well as AmmTX3. Synthetic peptides, native AaTX1 and rAmmTX3 peptides showed qualitatively the same pharmacological activity. Overall, these data identify a new biologically active toxin that belongs to a family of peptides active on Kv4.3 channel.

Abréviations: Aa, *Androctonus australis*; nAaTX1, native AaTX1; sAaTX1, synthetic AaTX1; sAmmTX3, synthetic AmmTX3; rAmmTX3, recombinant AmmTX3; FPLC, fast protein liquid chromatography; HPLC, high pressure liquid chromatography; TFA, tri fluoro acetic acid; CH_3CN, acetonitrile; BSA, bovine serum albumin.

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1. Introduction

Potassium channels enable the specific permeation of K^+ ions through cell membranes, and thus provide the basis for many important physiological processes, from modulation of electrical activity to regulation of signal transduction (Hille, 2001). Inherited disorders related to changes in excitability (episodic ataxia, epilepsy, heart arrhythmia) or to defects in sensory perception (hearing loss) have been associated with mutations in voltage activated potassium channel genes. Several potassium channels have been identified as interesting therapeutic targets (Shieh et al., 2000). Due to their high affinity and specificity, scorpion toxins provide powerful tools to study the structure and function of ion channels. They have been extensively exploited as molecular probes for mapping structure topologies and characterizing the pharmacological profiles of ion channels (Hidalgo and MacKinnon., 1995; MacKinnon et al., 1998; Rodríguez De La Vega et al., 2003). Scorpion toxins are composed of two major polypeptide populations: one consists of several classes of long-chain toxin affecting Na^+ channels, and the other one includes short-chain toxins affecting K^+, Ca^{2+} or Cl^- channels in excitable and non-excitable cell membranes. Most of these toxins share a common structural folding consisting of an alpha-helix connected by a network of three or four disulfide bridges to three β strands (Bontems et al., 1991). This Csαβ motif is a versatile scaffold that has been used through evolution to generate a tremendous diversity of sequences associated with specific biological activities (Zhu and Tytgat, 2004), leading to a combinatorial library of pharmacologically active peptides (Sollod et al., 2005). Scorpion toxins strongly alter channel function by binding to several receptor sites. From a pharmacological point of view, potassium channel toxins have been the most extensively studied. They have been classified into three subfamilies: α-, β- and γ-KTx (Tytgat et al., 1999). According to similarities in their sequences and specificity towards each corresponding subtype of K^+ channel, α-KTx channel toxins can be sorted into 26 molecular subfamilies (α-KTx1 to α-KTx26) (Tan et al., 2006; Mao et al., 2007). Pairwise sequence comparisons of these toxins showed that the α-KTx15 subfamily toxins are quite different from all the others (Pisciotta et al., 2000). These toxins were considered as revealing a new class of scorpion toxin binding sites in rat brain (Vacher et al., 2001, 2002). α-KTx15 toxins are Aa1, AaTX1 and AaTX2 from *Androctonus australis* (Piscsiotta et al., 1998, 2000; Legros et al., 2003); AmmTX3 and AmmTX from *Androctonus mauretanicus* (Vacher et al., 2002; Chen et al., 2005), BmTX3 and *BmKTX* from *Buthus martensi* (Huys et al., 2004); Tx406 from *Buthus occitanus,* (Kozminsky-Atias et al., 2007) and Discrepin from *Titius discrepans* (D'suze et al., 2004), scorpions venoms. Cited toxins are either purified from the crude venom or determined from cDNA library from telsons of their respective scorpion species. Only Aa1, AmmTX3, BmTX3 and Discrepin are characterized for their pharmacological activity and were found to block the IA currents (Prestipino G., 2009).

In this study, we purified AaTX1, which represents only 0.007% of Aa venom, and defined its pharmacological activity on I_A currents. The functional characterization was performed on substantia nigra dopaminergic neurons, as the effect of AmmTX3 on the I_A current was clearly defined in this preparation in a recent study (Amendola et al., 2012). Since AaTX1 is very minor component of Aa scorpion venom, we synthesized it as well as AmmTX3 (sAaTX1 and sAmmTX3, respectively), which differs by only one conserved amino acid (R/K) in the 19th position. We investigated their pharmacological activities.

2. Experimental procedures

2.1. Scorpion venom

Venom of *Androctonus australis (*Aa) scorpions from Beni Khedach (Tunisia) was collected by the veterinarian service of the Pasteur Institute of Tunisia and kept frozen at −20 °C in its crude form until use.

2.2. Animals

We used C57/BL6 mice (20 ± 2 g) provided by the veterinary service of the Pasteur Institute of Tunis for the *in vivo* toxicity test.

2.3. Purification

Crude venom was extracted with cold water (1:4 v/v), then centrifuged at 15 000 × g for 15 min. The supernatant was loaded on Sephadex G-50 gel filtration chromatography column (K26/100) equilibrated with 0.1 M acetic acid, to obtain mainly 5 fractions as previously described (Miranda et al., 1970). The major fraction named AahG50, is the toxic one and contains toxins of 3000 Da—7000 Da. After its lyophilization, the AaHG50 was fractionated by FPLC on a cation exchange Resource S pre-equilibrated with 0.05 M ammonium acetate pH 6.6. Proteins were eluted with a 40 min linear gradient from 0.05 to 0.5 M ammonium acetate, pH 6.6, at a flow rate of 1 ml/min. Absorbance was monitored at 280 nm.

HPLC purification of the fraction containing AaTX1 was performed using a C18 reversed-phase HPLC column (5 μm, 4.6 × 250 mm, Beckman), equipped with a Beckman Series 125 pump and a Beckman diode array detector set. Elution was controlled by means of the GOLD software. Proteins were eluted from the column at a flow rate of 0.8 ml/min, using a linear gradient (45 min) from 10 to 45 % of buffer B (0.1% TFA in CH_3CN) in buffer A (0.1% TFA in water). Polypeptide concentration was determined using QuantiPro BCA Assay Kit (Sigma—Aldrich).

2.4. Amino acid sequence determination and comparison with α-KTx15 toxins

Reduction with dithiothreitol and alkylation with 4-vinylpyridine, were performed as previously described (Srairi-Abid et al., 2000). The pyroglutamate residue was removed by pyroglutamate aminopeptidase: one unit hydrolyze 1 μmole of pyroglutamate aminopeptidase at 37 °C, pH 7. The N-terminal sequence of reduced, alkylated