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Tst26, a novel peptide blocker of Kv1.2 and Kv1.3 channels from the venom of *Tityus stigmurus*

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ABSTRACT

Using high-performance liquid chromatography Tst26, a novel potassium channel blocker peptide, was purified from the venom of the Brazilian scorpion *Tityus stigmurus*. Its primary structure was determined by means of automatic Edman degradation and mass spectrometry analysis. The peptide is composed of 37 amino acid residues and tightly folded through three disulfide bridges, similar to other K^+ channel blocking peptides purified from scorpion venoms. It contains the "essential dyad" for K^+ channel recognition comprised of a lysine at position 27 and a tyrosine at position 36. Electrophysiological assays revealed that Tst26 blocked hKv1.2 and hKv1.3 channels with high affinity ($K_d = 1.9$ nM and 10.7 nM, respectively) while it did not affect several other ion channels (mKv1.1, hKv1.4, hKv1.5, hERG, hIKCa1, hBK, hNav1.5) tested at 10 nM concentration. The voltage-dependent steady-state parameters of K^+ channel gating were unaffected by the toxin in both channels, but due to the fast association and dissociation kinetics Tst26 slowed the rate of inactivation of Kv1.3 channels. Based on the primary structure, the systematic nomenclature proposed for this peptide is α-KTx 4.6.

1. Introduction

Potassium channels are involved in numerous cellular functions such as the maintenance of resting membrane potential, control of cardiac and neuronal excitability, release of neurotransmitters, muscle contractility and hormone secretion. Pharmacological manipulation of these channels using blocking compounds is well established for therapeutic approaches in excitable cells, and results from the last few years with blockers of the voltage-gated Kv1.3 channel of lymphocytes have brought the prospect of achieving selective immunosuppression for the treatment of autoimmune diseases as well (Wulff et al., 2003). This led to increasing interest in peptides isolated from animal venoms capable of blocking Kv1.3 channels (Panyi et al., 2006).

Scorpion venoms contain a complex mixture of peptides, many of which can bind to ion channels with great affinity. Over 120 of these are known to block K^+ channels (reviewed in Rodriguez de la Vega and Possani, 2004), being more or less selective among different K^+ channels. Many of these toxins share a very similar structure having an α-helix and two β-strands linked by three disulfide bridges. A strategically positioned lysine and an aromatic residue nine positions downstream form the "functional dyad", which was also identified as a common feature of high affinity K^+ channel blocking scorpion

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peptides (Dauplais et al., 1997). The aromatic residue, however, is not a prerequisite for effective block of K^+ channels in general; it rather seems to determine selectivity among channel subtypes, such as Kv1.2 and Kv1.3 (Corzo et al., 2008). Moreover, scorpion toxins completely lacking this dyad, yet effectively blocking potassium channels also exist (Batista et al., 2002). Although the side chain of the dyad lysine that protrudes into the selectivity filter of K^+ channels is very important for high affinity block, a long lasting toxin-channel complex requires multiple contact points within the interacting surfaces (Aiyar et al., 1995). Differences in these contact points determine the selectivity of the toxin among different channels. High selectivity for one particular channel type is of utmost importance if a toxin or its derivative is to be used in therapy in the future to influence a specific function of the targeted cells (Kalman et al., 1998). Thus, the comparison of the selectivity of K^+ channel specific toxins with known structures is needed to identify key residues making contacts with the channels. This information, combined with molecular biological techniques then can be used to produce compounds that target K^+ channels with high affinity and selectivity to achieve the desired therapeutic effect (Panyi et al., 2006; Beeton et al., 2006).

Our group has purified and characterized several K^+ channel blocking toxins in the past (Peter et al., 2000; Péter et al., 2001; Bagdany et al., 2005; Olamendi-Portugal et al., 2005; Corzo et al., 2008). Here we describe the purification and characterization of a peptide obtained from the venom of the Brazilian scorpion *Tityus stigmurus*, named Tst26, and we report its effect on eight potassium channels and a cardiac sodium channel assayed by patch-clamp.

2. Materials and methods

2.1. Source of venom and chemicals

The venom of *T. stigmurus* was a gift from the Butantan Institute, Sao Paulo, Brazil. Phytohemagglutinin A and other chemicals for electrophysiology were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA and Budapest, Hungary). All solvents and chemicals used were analytical grade and double-distilled water was used throughout. For additional information see references (Batista et al., 2007; Barona et al., 2006).

2.2. Isolation procedures

The dried venom was solubilized in water and spun at $10{,}000 \times g$ for 5 min. One hundred μl containing 1.0 mg of the soluble venom was directly submitted to an analytical C18 reverse-phase column (dimensions of 250×10 mm) obtained from Vydac (Hesperia, CA, USA). Elution from the high-performance liquid chromatographer (HPLC) was obtained by using a linear gradient from 0% solution A [0.12% trifluoroacetic acid (TFA) in water] to 60% solution B (0.10% TFA in acetonitrile), run for 60 min. The detection was monitored by absorbance at 230 nm with 0.5-unit sensitivity and eluted at 1 ml/min flow-rate. Fractions were collected manually and dried using a Savant Speed-Vac drier. Some sub-fractions were further separated using analytical columns in the same system, but slightly different gradients as described below (see text and figure legends).

2.3. Amino acid sequence determination and mass spectrometry analysis

Amino acid sequence determination of pure peptide was performed by automatic Edman degradation in a Beckman LF 3000 Protein Sequencer (Palo Alto, CA, USA) using the chemicals and procedures previously described (Batista et al., 2004; Barona et al., 2006).

A sample of the homogeneous peptide was reconstituted to a final concentration of 0.1–0.5 μg/μl of 50% acetonitrile with 1% acetic acid and directly applied into a Finnigan LCQ ion trap mass spectrometer (San Jose, CA, USA), using a Surveyor MS syringe pump delivery system. The experimental procedures followed for the mass spectrometry determinations and analysis performed with Tst26, including the LC–MS/MS fragmentation, are the same as earlier described by our group in (Batista et al., 2007).

2.4. Cells

2.4.1. Lymphocyte separation

Kv1.3 currents were measured in human peripheral T lymphocytes. Heparinized human peripheral venous blood was obtained from healthy volunteers. Mononuclear cells were separated by Ficoll–Hypaque density gradient centrifugation. Collected cells were washed twice with Ca^{2+} and Mg^{2+} free Hank's solution containing 25 mM HEPES buffer (pH 7.4). Cells were cultured in a 5% CO_2 incubator at 37 °C in 24-well culture plates in RPMI-1640 supplements with 10% FCS (Sigma–Aldrich, Hungary), 100 μg/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine at 0.5×10^6/ml density for 3–4 days. The culture medium also contained 2.5 or 5 μg/ml of phytohemagglutinin A (PHA-P, Sigma–Aldrich Kft, Hungary) to increase K^+ channel expression (Deutsch et al., 1986).

2.4.2. Heterologous expression of channels

Cos-7 or tsA-201 cells were transiently transfected with the plasmid for hIKCa1 (subcloned into the pEGFP-C1 (Clontech) in frame with green fluorescent protein (GFP), a gift of H. Wulff, UC Davis, CA, USA); or co-transfected with plasmids for GFP and for hKv1.2 (pcDNA3/Hygro vector containing the full coding sequence for Kv1.2, a gift from S. Grissmer, University of Ulm); or hKv1.4 (hKv1.4ΔN: the inactivation ball deletion mutant of Kv1.4, a gift from D. Fedida, University of British Columbia, Vancouver, Canada); or hNav1.5 (a gift from R. Horn, Thomas Jefferson University, Philadelphia, PA,USA). hBK (hSlo1 gene (U11058), in pCI-neo plasmid, a gift from T. Hoshi, University of Pennsylvania, Philadelphia, PA) and hKv1.5 (a gift from D. Fedida, University of British Columbia, Vancouver, Canada) channels were transiently co-transfected into tsA-201 cells along with plasmids encoding GFP.

Transfections were done at a GFP:channel DNA molar ratio of 1:5 using Lipofectamine 2000 reagent according to manufacturer's protocol (Invitrogen, Carlsbad, CA, USA),