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Jingzhaotoxin-XII, a gating modifier specific for Kv4.1 channels ☆

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Abstract

Jingzhaotoxin-XII (JZTX-XII), a 29-residue polypeptide, was purified from the venom of the Chinese tarantula *Chilobrachys jingzhao*. Electrophysiological recordings carried out in *Xenopus laevis* oocytes showed that JZTX-XII is specific for Kv4.1 channels, with the IC_{50} value of 0.363 μM. It interacts with the channels by modifying the gating behavior. JZTX-XII shares 80% sequence identity with phrixotoxin1, a potent inhibitor for Kv4.2 and Kv4.3 channels. Structure analysis indicates that the difference of the charge distribution in the interactive surface perhaps influences the specific pharmacology of the toxins. JZTX-XII should be a valuable tool for the investigation of the Kv4.1 channels.

Keywords: Spider; Gating modifier; Kv4.1 channel; Kv2.1 channel

1. Introduction

The *shal* subfamily comprises three distinct genes, Kv4.1, Kv4.2, and Kv4.3, that all express transient K^+ currents in the nervous system and the heart (Baldwin et al., 1991; Pak et al., 1991; Serodio and Rudy, 1998). The proteins encoded by these genes are highly homologous within the transmembrane regions, with divergent amino and carboxy termini (Birnbaum et al., 2004). The Kv4.2 and Kv4.3 channels are expressed in a variety of tissues, with particularly high levels in the brain and heart (Serodio et al., 1994, 1996), but Kv4.1 channels are expressed in the brain, and appear to be quite low compared with Kv4.2 and Kv4.3 expression in the CNS. In the nervous system, Kv4 channels prevent back-propagating action potentials, help to establish slow repetitive spike firing and contribute to spike repolarization and signal amplification (Hoffman et al., 1997; Shibata et al., 2000). In the heart, these channels are mainly responsible for the rapid initial phase of repolarization of the cardiac action potential (Fedida and Giles, 1991). Thus, the *Shal*-type channels are implicated in membrane excitability, synaptic transmission, and repolarization of cardiac myocytes.

☆ *Ethical statement*: All the animals were used in adherence with protocols approved by the Hunan Normal University Animal Care and Use Committee.

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Spider venom is a unique source of toxins for *Shal*-type channels. Heteropodatoxins, Jingzhaotoxin-V, and phrixotoxins are short peptides, highly reticulated by disulfide bridges, which are potent Kv4.2 and Kv4.3 channel inhibitors (Zeng et al., 2007; Diochot et al., 1999; Sanguinetti et al., 1997). HmTx and ScTx, isolated from the venom of the African tarantulas, exhibit different affinities to Kv2.1 and Kv4 channels (Escoubas et al., 2002). These toxins modify the kinetics of either inactivation or activation gating through interaction with the voltage-sensing domain of Kv channels (Zarayskiy et al., 2005).

In the present work, a specific Kv4.1 inhibitor named Jingzhaotoxin-XII (JZTX-XII) was isolated from the venom of the Chinese tarantula *Chilobrachys jingzhao*, which shares 80% sequence similarity with PaTxl. Structure analysis provided significant information of the toxin selectivity for potassium channels. JZTX-XII should be a valuable tool for the study of the Kv4.1 channels.

2. Materials and methods

2.1. Toxin purification and sequencing

JZTX-XII was purified from Chinese tarantula *C. jingzhao* venom using a combination of ion-exchange chromatography and reverse-phase high-pressure liquid chromatography (HPLC) as described previously (Xiao et al., 2005). The molecular mass was determined using a Voyager-DE^{TM} STR MALDI-TOF mass spectrometer of ABI Company. Saturated solution of α-cyano-4-hydroxy-cinnamic (CCA) in ACN/water (1:1) was used as the matrix. The entire amino acid sequence was obtained by automated Edman degradation using an Applied Biosystems 491 pulsed-liquid-phase sequencer from Applied Biosystem Inc.

2.2. Homology modeling

The basic local alignment search tool (BLAST) was applied to screen the non-redundant NCBI database (http://www.ncbi.nlm.nih.gov) for sequences with homology with the sequence of JZTX-XII. Among the candidates, the sequence of PaTx1 shares a high similarity with that of JZTX-XII. We modeled the structure of JZTX-XII on the basis of the NMR structure of PaTx1 (PDB entry 1V7F). Structure modeling and visualization were done with Insight II software on a Silicon Graphics workstation. Energy minimization and simulated annealing cycles were run (Wang et al., 2006).

2.3. Electrophysiological recordings

Capped cRNAs encoding ion channels were synthesized after linearizing the plasmids and performing the transcription by a standard protocol (Krieg and Melton, 1987). For in vitro transcription, the plasmids pCI containing the genes for Kv4.1 (Isbrandt et al., 2000), Kv2.1 (Albrecht et al., 1993), and Kv1.1 (Klumpp et al., 1991) were first linearized with *NotI*; the plasmid PcDNA3 containing the gene for Kv1.2 (Adda et al., 1996) was linearized with *SphI*; the plasmid pCI-neo containing the gene for Kv1.3 was linearized with *NotI* (Attali et al., 1992); the plasmid PcDNA3.1containing the gene for Kv4.2 was linearized with *SmaI* (Serodio et al., 1994); the plasmids pSP64 containing the genes for Kv1.4 (Rasmusson et al., 1995) and Kv3.1 (Yue et al., 2000) were linearized with *EcoRI*. Using the linearlized plasmids as templates, cRNAs encoding Kv1.4 and Kv3.1 channels and cRNAs encoding the other channels were synthesized in vitro using the large-scale SP6 or T7 mMESSAGE mMACHINE transcription kit, respectively (Ambion, USA).

Stage IV-VI *Xenopus laevis* oocytes were collected from mature female *X. laevis* under anaesthesia by putting on ice. Then the oocytes were defolliculated by treatment with 1 mg/ml collagenase in calcium-free ND96 solution (pH 7.5) containing concentrations of (in mM) NaCl 96, KCl 2, $MgCl_2$ 1, and HEPES 10. The isolated oocytes were incubated in OR_2 solution (pH = 7.5) at 18 °C for microinjection. OR_2 solution contains (in mM) NaCl 82.5, KCl 2.5, $CaCl_2$ 1, Na_2HPO_4 1, $MgCl_2$ 1, HEPES 5, supplemented with 50 mg/l gentamycin sulfate (only for incubation).

Oocytes were injected with 41 nl of 100–500 ng/μl cRNA using a microprocessor-controlled nanoliter injector (WPI, USA). Whole-cell currents were recorded from oocytes incubated for 2–5 days after microinjection using the two-microelectrode voltage-clamp (TURBO TEC-03X, NPI Electronic, Germany). Voltage and current electrodes (0.1–1 MΩ) were filled with 3 M KCl. Oocytes were studied in a recording chamber that was perfused with an extracellular solution containing (in mM): RbCl 50, NaCl 50, $MgCl_2$ 1, $CaCl_2$ 0.3, and HEPES 5, pH 7.5 with NaOH (only for recording Kv2.1 currents), or OR_2 solution. Current records were