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Heteropoda toxin 2 is a gating modifier toxin specific for voltage-gated K^+ channels of the Kv4 family

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Abstract

Kv4 voltage-gated K^+ channels are responsible for transient K^+ currents in the central nervous system and in the heart. HpTx2 is a peptide toxin that selectively inhibits these currents; making it a useful probe for understanding Kv4 channel structure and drug binding. Therefore, we developed a method to produce large amounts of recombinant HpTx2. Recombinant toxin inhibits all three Kv4 isoforms to the same degree; however, the voltage-dependence of inhibition is less apparent for Kv4.1 than for Kv4.3. Similarly, recombinant $HpTx2_{GS}$ effects gating characteristics of both channels, but Kv4.1 to a much lesser degree. The toxin lacks affinity for Kv1.4, Kv2.1, and Kv3.4. To locate the binding site, the amino acids linking the third and forth membrane spanning segments of Kv4.3 were replaced with analogous amino acids of Kv1.4. The chimeric K^+ channel was completely insensitive to block by rHpTx2, suggesting that its binding site is near the channel's voltage sensor. These data show that $rHpTx2_{GS}$ is a gating modifier toxin that binds to a site remote from the pore.
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1. Introduction

Voltage-dependent potassium channels are a widely distributed and diverse group of proteins with critical roles in maintenance of membrane resting potential, action potential repolarization, and signal transduction. Among voltage-gated K^+ channels, the Kv4 family has drawn considerable interest because of its important functions in the heart and central nervous system. In the heart, Kv4 channels are responsible for the fast recovering transient outward current (I_{to}), which is critical in determining the shape and duration of early repolarization in the cardiac action potential (Strauss et al., 2001). In the central nervous system, Kv4 channels are found in the somatodendritic region of neurons, where they control the frequency of repetitive spike firing and back propagation of action potentials (Birnbaum et al., 2004).

Abbreviations: HpTx2, Heteropoda toxin 2; $rHpTx2_{GS}$, recombinant HpTx2; ICK, inhibitor cystine knot; I_{to}, cardiac transient outward K^+ current; NMR, nuclear magnetic resonance; OD_{600}, optical density at 600 nm; EDTA, ethylene diamine tetra acetate; IMAC, immobilized metal affinity chromatography; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid; HaTx1, Hanatoxin 1; HPLC, high pressure liquid chromatography.

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There are three members of the Kv4 family (Kv4.1, Kv4.2, and Kv4.3) that are encoded by separate genes (*KCND1-3* in humans) (Isbrandt et al., 2000); Kv4.3 has two alternatively spliced forms varying by 19 amino acids (Ohya et al., 1997). The electrophysiological properties of Kv4 channels are similar; all have fast activation and rapid inactivation with multiple time constants. They are members of the *Shaker*-related super family of voltage-gated ion channels. As such, they have six membrane spanning segments, designated S1 through S6, with the selectivity filter and pore found between S5 and S6, and the voltage sensor in S4 (Fig. 1, top). Four subunits are required to form the symmetric pore (Yellen, 2002). Like many channels, Kv4s are complexes with an array of ancillary subunits, including KChIPs, DPPX, and PSD domain proteins (Birnbaum et al., 2004), many of which have been shown to alter electrophysiological properties. This has made identification and study of native channel protein complexes difficult.

Peptide toxins purified from a variety of species have been invaluable tools for the study of ion channel proteins. One diverse group of toxins are peptides of 29–40 amino acids with three disulfide bonds that form an 'inhibitor cystine knot' (ICK) motif (Norton and Pallaghy, 1998). Amongst all well studied peptide toxins, direct occlusion of the channel pore is the most common mechanism of block (MacKinnon and Miller, 1988). However, many well studied ICK toxins inhibit ion currents through interference with the channel's gating mechanism (Norton and Pallaghy, 1998).

While ICK toxins can block voltage-gated Na^+ or Ca^{2+} channels, at least 15 toxins apparently containing an ICK motif have been shown to bind either or both Kv2 or Kv4 K^+ channel families. One of these was the second of three purified from the venom of the huntsman spider *Heteropoda venatoria* (Sanguinetti et al., 1997), HpTx2. Native HpTx2, and the closely related HpTx3 block I_{to} in hearts from rats, ferrets, and mice (Brahmajothi et al., 1999; Guo et al., 1999; Kassiri et al., 2002; Sanguinetti et al., 1997), while having no apparent effect on K^+ currents not based on Kv4 channels (Brahmajothi et al., 1999; Sanguinetti et al., 1997). As expected, the native toxin inhibited Kv4.2, the molecular substrate of rat I_{to}. Inhibition was voltage-dependent, with much less block at more positive potentials. Shifts in steady-state activation and inactivation suggested that HpTx2 was a 'gating-modifier' toxin, similar to Hanatoxin (HaTx) (Swartz and MacKinnon, 1995; Sanguinetti et al., 1997). NMR determination of the tertiary structure of a recombinant form of HpTx2 showed that it is a member of the ICK family (Bernard et al., 2000). These authors suggested the possibility that rHpTx2 is not a gating modifier, but a pore-blocker, based on a comparison of the toxin's dipolar moment to known pore blocking toxins.

We report a novel method of *E. coli* synthesis of a recombinant form of HpTx2. The recombinant toxin, $rHpTx2_{GS}$, inhibits all three Kv4 channels. Inhibition of Kv4.3 and Kv4.2 is strongly voltage-dependent, while inhibition of Kv4.1 shows less voltage-dependence. The toxin lacks affinity for Kv1.4, Kv3.4, and most importantly, Kv2.1. Application of $rHpTx2_{GS}$ to *Xenopus* oocytes expressing Kv4.3 show depolarizing shifts and decreasing slope factors of steady-state activation and inactivation relationships. Recombinant $HpTx2_{GS}$ also speeds up Kv4.3 inactivation. The voltage-dependent inhibition and the effects of the toxin on Kv4.3 kinetics suggest that $rHpTx2_{GS}$ is a gating modifier toxin. A mutant Kv4.3 that replaced the S3–S4 linker region of Kv4.3 with that of a channel with no affinity for $rHpTx2_{GS}$ was not affected by toxin application. Since the linker region is close to the S4 voltage-sensor, but remote from the channel pore, these data strongly suggest that $rHpTx2_{GS}$ is a gating modifier toxin.

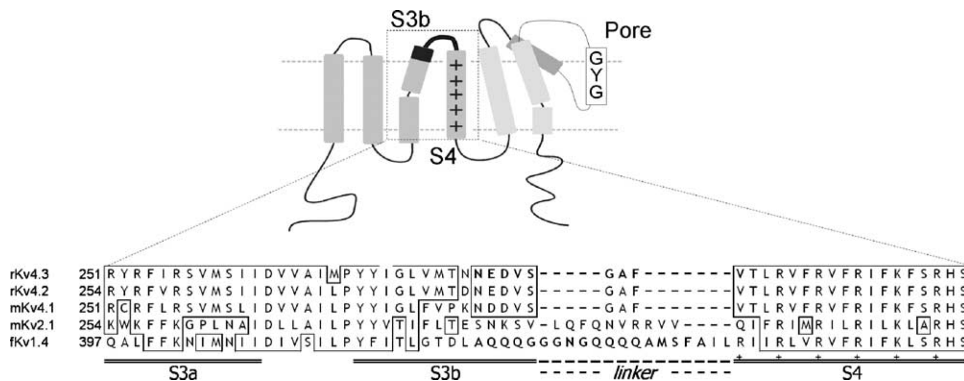

Fig. 1. Alignment of K^+ channel S3–S4 regions. The top panel shows a schematic of the six membrane-spanning structure of a single voltage-gated K^+ channel α subunit; the membrane spanning regions S3a, S3b and S4 assignments were made as described (Lee et al., 2003; Li-Smerin and Swartz, 2001). The bottom panel is the alignment of some mammalian voltage-gated K^+ channel α subunits. The amino acids in bold in Kv4.3 and Kv1.4 are those that were substituted in pKv43 [S34]. The boxes surround identical amino acids, and those with very similar physical properties. The double lines highlight S3a, S3b, and S4, respectively. The dashed lines denote the S3–S4 linker. The dashed box and lines in the top panel highlight the region of the channel aligned at the bottom.

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