

Inhibition of neuronal tetrodotoxin-sensitive Na⁺ channels by two spider toxins: hainantoxin-III and hainantoxin-IV

Yucheng Xiao, Songping Liang*

College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, People's Republic of China

Received 13 May 2003; received in revised form 25 July 2003; accepted 29 July 2003

Abstract

Hainantoxin-III and hainantoxin-IV, isolated from the venom of the Chinese bird spider *Seleconosmia hainana*, are neurotoxic peptides composed of 33–35 residues with three disulfide bonds. Using whole-cell patch-clamp technique, we investigated their action on ionic channels of adult rat dorsal root ganglion neurons. It was found that the two toxins did not affect Ca²⁺ channels (both high voltage activated and low voltage activated types) nor tetrodotoxin-resistant voltage-gated Na⁺ channels (VGSCs). However, hainantoxin-III and hainantoxin-IV strongly depressed the amplitude of tetrodotoxin-sensitive Na⁺ currents with IC₅₀ values of 1.1 and 44.6 nM, respectively. Both hainantoxin-III (1 nM) and hainantoxin-IV (50 nM) caused a hyperpolarizing shift of about 10 mV in the voltage midpoint of steady-state Na⁺ channel inactivation, but they showed difference in the repriming kinetics of VGSCs: hainantoxin-III significantly decreased the recovery rate from inactivation at a prepulse potential of –80 mV while hainantoxin-IV did not do. It is interesting to note that similar to huwentoxin-IV, the two hainantoxins did not affect the activation and inactivation kinetics of Na⁺ currents and at a concentration of 1 μM they completely inhibited the slowing inactivation currents induced by BMK-I (toxin I from the scorpion *Buthus martensi* Karsch), a scorpion α-like toxin. The results indicate that hainantoxin-III and hainantoxin-IV are novel spider toxins and affect the mammalian neural Na⁺ channels through a mechanism quite different from other spider toxins targeting the neural receptor site 3, such as δ-aractoxins and μ-agatoxins.

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Keywords: Spider toxin; Dorsal ganglion neuron; Na⁺ current; Patch-clamp, whole-cell

1. Introduction

Voltage-gated Na⁺ channels (VGSCs) are transmembrane proteins present in most neurons and muscle cells. Their opening constitutes the depolarization phase of the action potential. Therefore, they are major contributors to the generation and propagation of electrical signals in neurons and contraction of muscles. The activity of VGSCs can be modulated with different mechanisms by many toxins purified from the venoms of different animals such as snakes, scorpions, marine snails and spiders. These native toxins, especially tetrodotoxin and scorpion α, β-toxins, are important useful tools for studying the structure–function relationship of Na⁺ channels. To date at least six sites (1–6) on neural Na⁺ channels have been disclosed by using these toxins (Cestele et al., 1995), and when those toxins bind at

the six sites selectively, the characterizations of channels show some main corresponding changes: blocking channel conductance (site 1 toxins), causing persistent activation (site 2 toxins), slowing channel inactivation (site 3 toxins), induction of both a shift in the voltage dependence of inactivation and a reduction of the peak currents (site 4 toxins), shifting inactivation to more negative membrane potential and blocking inactivation (site 5 toxins) and voltage-independent inhibition of the inactivation of currents (site 6 toxins). Although several toxins may target the same site, they do not bind to the same active residues in Na⁺ channel proteins. For example, single site mutation demonstrates that tetrodotoxin has stronger interaction with Tyr401 than saxitoxin, another site 1 toxin, in the adult skeletal muscle Na⁺ channels (Penzotti et al., 1998). Furthermore, native toxins provide a key for further discriminating pharmacologically among different Na⁺ channel subtypes. VGSCs can be classified into tetrodotoxin-sensitive and tetrodotoxin-resistant types (Ogata and Tatebayashi, 1993). The tetrodotoxin-sensitive Na⁺ channels can be divided into three subtypes by μ-conotoxins (Shon et al.,

* Corresponding author. Tel.: +86-731-8872556; fax: +86-731-8861304.

E-mail address: liangsp@public.cs.hu.cn (S. Liang).



Fig. 1. Comparison of the primary structures of three spider toxins: huwentoxin-IV, hainantoxin-III and hainantoxin-IV. Note the amidated C-terminals. Huwentoxin-IV was isolated from the venom of the Chinese bird spider *Selenocosmia huwena* and hainantoxin-III and hainantoxin-IV were isolated from another Chinese bird spider *Selenocosmia hainana*. Hainantoxin-III and hainantoxin-IV shared 30% and 80% sequence identity with huwentoxin-IV, respectively. The identical residues are shaded in black.

1998): (1) sensitive to μ -PIIIA and μ -conotoxin GIIIA, (2) sensitive to μ -PIIIA but not to μ -GIIIA, and (3) resistant to μ -PIIIA and μ -GIIIA.

Both hainantoxin-III (P83464, <http://www.pubmed.com>) and hainantoxin-IV are isolated from the venom of the Chinese bird spider *Seleconosmia hainana* (Liang et al., 1999). Their sequences have been determined to be 33–35 residues with three disulfide bonds and their C-terminal ends are amidated (Fig. 1). The intraperitoneal LD₅₀ values of hainantoxin-IV to mice is 0.2 mg/kg. It can block

neuromuscular transmission in the isolated nerve-synapse preparations of mouse phrenic nerve-diaphragm (Liu et al., 2002). However, the mechanisms of neuromuscular block are still unknown. In this report, we describe the effect of the two spider toxins on the ionic channels in adult rat dorsal root ganglion cells.

2. Materials and methods

2.1. Purification of toxin

Hainantoxin-III and hainantoxin-IV were purified using reverse phase high-performance liquid-phase chromatograph (HPLC) followed by ion-exchange chromatograph as described earlier in our laboratory (Liang et al., 1999).

2.2. Cell isolation procedures

Rat dorsal root ganglion neurons were acutely dissociated and maintained in a short-term primary culture using the method described by Wang et al. (1998). Briefly, 30-day

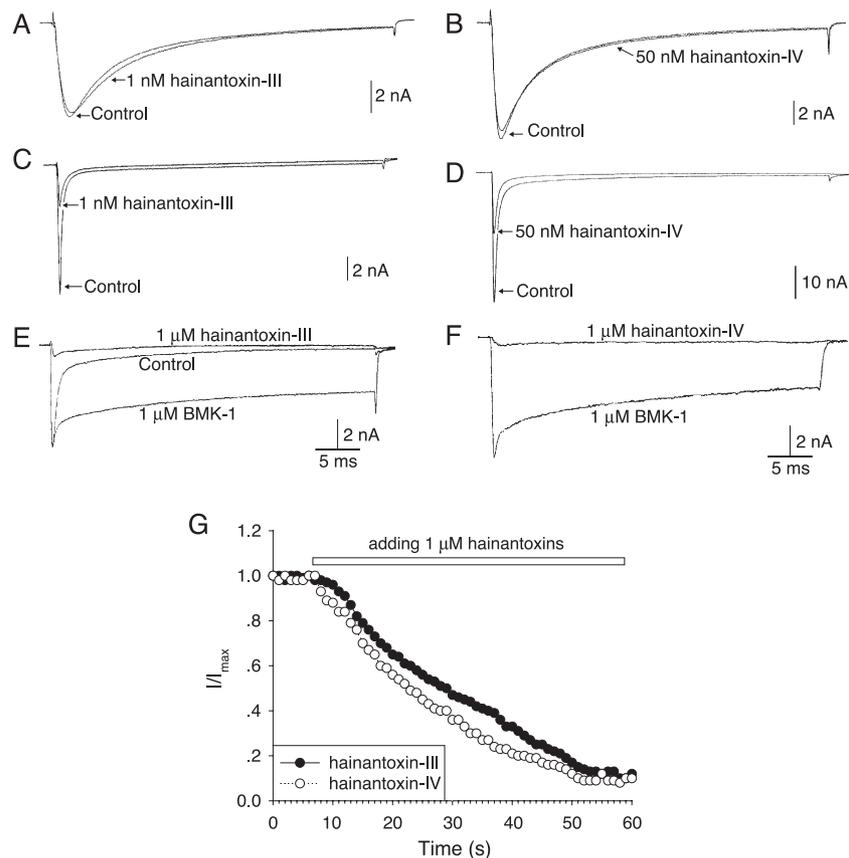


Fig. 2. Effects of hainantoxin-III and hainantoxin-IV on the tetrodotoxin-resistant (A, B) and tetrodotoxin-sensitive (C–G) Na⁺ currents on rat dorsal root ganglion neurons. Both kinds of currents were induced by a 50-ms depolarizing potential of -10 mV from a holding potential of -80 mV. (C, D) Effects of tetrodotoxin-sensitive currents: (C) 1 nM hainantoxin-III and (D) 50 nM hainantoxin-IV. The above results suggested that the two toxins have no effect on tetrodotoxin-resistant Na⁺ currents while they inhibit tetrodotoxin-sensitive Na⁺ currents by $49.1 \pm 5.5\%$ (hainantoxin-III) and $57.2 \pm 5.4\%$ (hainantoxin-IV). The slowing tetrodotoxin-sensitive currents induced by 1 μ M BMK-I were inhibited by 1 μ M hainantoxin-III (E) and 1 μ M hainantoxin-IV (F). (G) The time course of inhibiting tetrodotoxin-sensitive currents in the presence of 1 μ M hainantoxin-III (●) or 1 μ M hainantoxin-IV (○).

adult Sprague–Dawley rats of either sex were killed by decapitation without anesthetization, the dorsal root ganglia were removed quickly from the spinal cord, and then they were transferred into Dulbecco's modified eagle's medium (DMEM) containing trypsin (0.5 g/l, type III), collagenase (1.0 g/l, type IA) and DNase (0.1 g/l, type III) to incubate at 34 °C for 30 min. Trypsin inhibitor (1.5 g/l, type II-S) was used to terminate enzyme treatment. After transfer into 35-mm culture dishes (corning, Sigma) which contained 95% DMEM, 5% newborn calf serum, hypoxanthine aminopterin thymidine supplement and penicillin–streptomycin, the dorsal root ganglion cells were incubated in CO₂ incubator (5% CO₂, 95% air, 37 °C) for 1–4 h before patch-clamping.

2.3. Electrophysiological recordings

Macroscopic currents (filtered at 10 kHz, digitized at 3 kHz with a EPC-9 patch-clamp amplifier, HEKA Electronics, Germany) were recorded at room temperature (20–25 °C). Micropipettes (2–3 μm diameter) were pulled from borosilicate glass capillary tubing by using a two-step vertical puller (PC-10, Narishige, Olypmus) and heat-polished with a microforge (MF-900, Narishige). The resistances of micropipettes were 1–2 MΩ after being filled with internal solution. Ca²⁺ currents were recorded using an internal solution containing (in mM): CsCH₃SO₃ 108, MgCl₂ 4, EGTA 9, Hepes 9, MgATP 3.6, creatine phosphate (Tris salt) 14, GTP (Tris salt) 1, creatine kinase at 50 units/ml at pH 7.4, and the external bathing solution contained (in mM): BaCl₂ 5, tetraethylammonium chloride 160, EGTA 0.1, HEPES 10 at pH 7.4 (Mintz et al., 1991). Na⁺ currents were recorded using an internal solution containing (mM): CsF 135, NaCl 10, Hepes 5 at pH 7.0 and the external bathing solution contained (mM): NaCl 30, CsCl 5, D-glucose 25, MgCl₂ 1, CaCl₂ 1.8, HEPES 5, tetraethylammonium chloride 20, tetramethylammonium chloride 70 at pH 7.4 (Zeto et al., 2000). An Ag–AgCl pipette/150 mM NaCl–agar bridge was introduced between bath electrode and bathing solution to avoid disturbing the composition of the external solution. After establishing the whole-cell recording configuration, the cell was allowed to stabilize for a period of more than 3–4 min to allow adequate equilibration between the micropipette solution and the cell interior. The needed concentrations of hainantoxins were dissolved in Na⁺ or Ca²⁺ external solution and then about 10 μl volume was applied to the experimental cells by pressure injection with a microinjector (IM-5B, Narishige).

2.4. Data analysis

Data analysis was performed using Pulsefit (HEKA Electronics) and Sigmaplot (Sigma, USA). All data are presented as mean ± standard error and *n* is the number of independent experiments.

The fitted curves of both concentration-dependent inhibition (Inhibition%) and steady-state Na⁺ channel inactivation (*I/I*_{max}) were obtained by using the following form of the Boltzmann equation:

Inhibition% = 100/[1 + exp(*C* – IC₅₀)/*k*] (1)

$$I/I_{\max} = 1/[1 + \exp(V - V_{1/2})/k] \quad (2)$$

In Eq. (1) where IC₅₀ is the concentration of toxin at half-maximal inhibition and *k* is the slope factor, *C* is the toxin concentration. In Eq. (2) where *V*_{1/2} is the voltage of half inactivation and *k* is the slope factor, *V* is the test voltage.

3. Results

3.1. Effects of toxins on voltage-gated Na⁺ currents

Dorsal root ganglion cells with diameters of 20–40 μm were selected to study Na⁺ channels, for larger dorsal root ganglion cells from older animals tend to express fast tetrodotoxin-sensitive Na⁺ currents while smaller ones (<10 μm) tend to express slow tetrodotoxin-resistant Na⁺ currents (Su et al., 1997). Tetrodotoxin (200 nM) was added to the external bathing solution to separate tetrodotoxin-resistant Na⁺ currents from tetrodotoxin-sensitive Na⁺ currents. Under voltage-clamp conditions, both kinds of Na⁺ currents on dorsal root ganglion cells were elicited by a 50-ms step depolarization to –10 mV from a holding potential of –80 mV every second. Hainantoxin-III (1 nM) and hainantoxin-IV (50 nM) did not significantly affect tetrodotoxin-resistant currents (Fig. 2). However, the other kind of Na⁺ current was sensitive to the two hainantoxins. At the same concentration, hainantoxin-III and hainantoxin-IV reduced the peak amplitude of control tetrodotoxin-sensitive Na⁺ currents to a maximum effect by 49.1 ± 5.5% (*n* = 12)

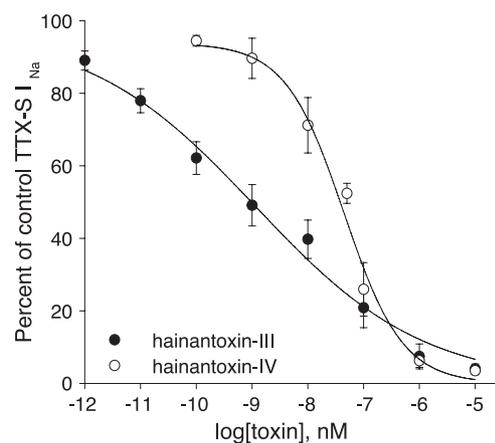


Fig. 3. The concentration-dependent block of tetrodotoxin-sensitive Na⁺ currents on rat dorsal root ganglion neurons. Every data point (mean ± S.E.) which came from 5 to 14 cells shows current relative to control. Both data points of hainantoxin-III (○) and hainantoxin-IV (●) were fitted according to Boltzmann equation (Eq. (1)) (see Materials and methods). The above results indicate that the IC₅₀ values of hainantoxin-III and hainantoxin-IV are 1.1 and 44.6 nM, respectively.

and $57.2 \pm 5.4\%$ ($n=8$) on dorsal root ganglion neurons, respectively. The effects of inhibition were rapid and $1 \mu\text{M}$ hainantoxin could completely inhibit tetrodotoxin-sensitive Na^+ currents within less than 1 min ($n=5$, Fig. 2G). It was observed that unlike other spider toxins (e.g. δ -aractoxins), after being blocked by hainantoxins, the shape of Na^+ currents was similar to that of control, indicating that two spider toxins did not change the activation and inactivation kinetics of tetrodotoxin-sensitive Na^+ currents. In order to determine that two hainantoxins did not act on site 3, we further observed the interaction between them and site 3 toxins. BMK-I, acting on site 3, is a typical α -like toxin isolated from the venom of the Asian scorpion, *Buthus martensi* Karsch (Ji et al., 1996). BMK-I ($1 \mu\text{M}$) did not affect the amplitude of peak tetrodotoxin-sensitive Na^+ currents, but it slowed the currents' inactivation by $71.1 \pm 6.4\%$ ($I_{\text{inac}}/I_{\text{peak}}$). Hainantoxin-III ($1 \mu\text{M}$) and hainantoxin-IV ($1 \mu\text{M}$) completely inhibited the inactivation currents induced by BMK-I ($n=6$, Fig. 2E,F), suggesting that both spider toxins modulated tetrodotoxin-sensitive Na^+

channels through a mechanism distinct from BMK-I. The reductions of hainantoxin-III and hainantoxin-IV were concentration-dependent with the IC_{50} value of 1.1 and 44.6 nM, respectively (Fig. 3). When the dose curves were fitted to Boltzmann equation (see Materials and methods), the slope factor (k) of hainantoxin-III (-1.51 ± 0.31 nM) was larger than that of hainantoxin-IV (-0.53 ± 0.12 nM).

3.2. Effects of toxins on the activation and inactivation kinetics of voltage-gated Na^+ channel

Fig. 4 shows the current–voltage (I – V) curves of both tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels which yield initial activated voltage, reversal potential and the active voltage of peak inward currents. When dorsal root ganglion cells were held at the membrane rest potential -80 mV, under control conditions tetrodotoxin-sensitive Na^+ currents were initially elicited at -50 mV, reached maximal amplitude at around -20 mV and reversed at about $+35$ mV while tetrodotoxin-resistant currents were at

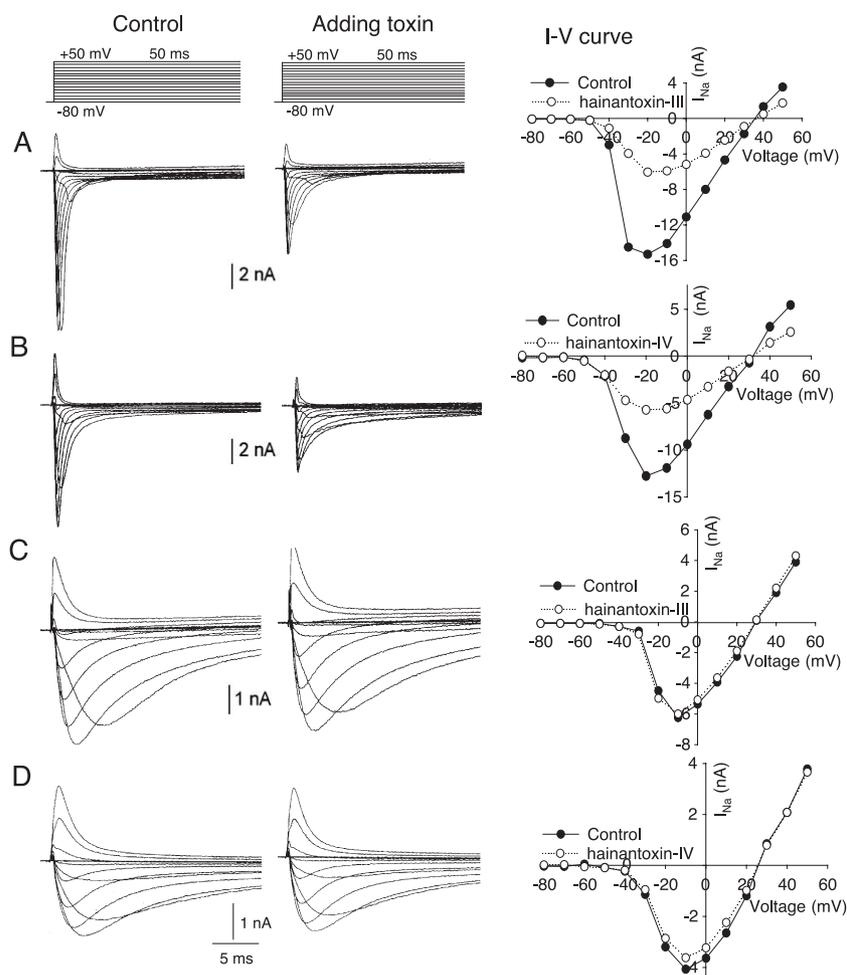


Fig. 4. Effects of hainantoxin-III (1 nM) and hainantoxin-IV (50 nM) on the current–voltage relationships of two kinds of Na^+ channels on rat dorsal root ganglion neurons. The groups of both tetrodotoxin-sensitive (A, B) and tetrodotoxin-resistant (C, D) currents were induced by 50-ms depolarizing steps to various potentials from a holding potential of -80 mV. Test potentials ranged from -80 to $+50$ mV. The current–voltage (I – V) curve (right) of Na^+ currents showed the relationships between current traces before (left) and after (middle) adding 1 nM hainantoxin-III (A, C) or 50 nM hainantoxin-IV (B, D).

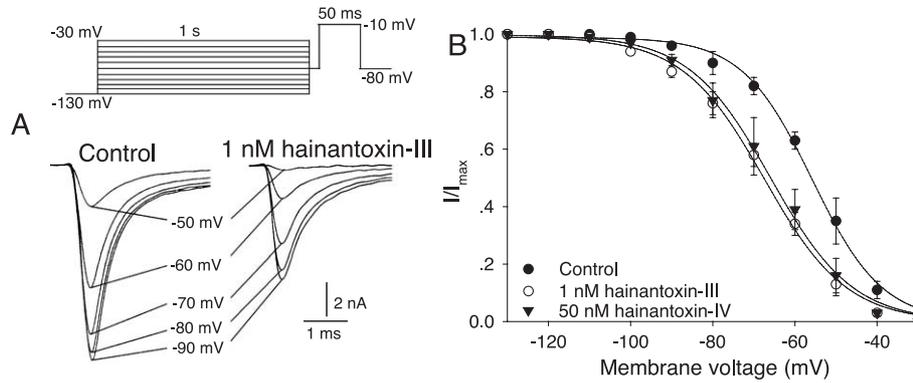


Fig. 5. Effects of hainantoxin-III and hainantoxin-IV on the steady-state inactivation of Na^+ channels on rat dorsal root ganglion neurons. Tetrodotoxin-sensitive currents were induced by a 50-ms depolarizing potential of -10 mV from various prepulse potentials for 1 s which ranged from -130 to -20 mV with a 10 mV increment. (A) Typical current traces were obtained following a 50-ms depolarization to -10 mV from prepulse potential of -90 , -80 , -70 , -60 and -50 mV. (B) The dots of 1 nM hainantoxin-III (\circ) and 50 nM hainantoxin-IV (\bullet) were fitted according to the Boltzmann equation (Eq. (2)) (see Materials and methods). Under control conditions ($n=14$), 1 nM hainantoxin-III ($n=7$) and 50 nM hainantoxin-IV ($n=5$), the $V_{1/2}$ were -55.8 ± 0.56 , -67.4 ± 0.62 and -65.9 ± 0.72 mV, respectively, and the k were -8.54 ± 0.48 , -10.0 ± 0.58 and -10.0 ± 0.62 mV, respectively.

about -40 , -10 and $+25$ mV, respectively. After 1 nM hainantoxin-III and 50 nM hainantoxin-IV treatment for 2 min, the inhibition of currents could be observed at all test pulses, but the two spider toxins did not change the threshold of activation and the activation voltage of inward currents. There was also no shift in the membrane reversal potential, implying that it did not change the ion selectivity of channels. Like other spider toxins, hainantoxin-III and hainantoxin-IV did not affect the $I-V$ curves of tetrodotoxin-resistant Na^+ currents. Therefore, both of them were without effect to tetrodotoxin-resistant Na^+ channels and did not alter the steady-state activation and inactivation kinetics of tetrodotoxin-resistant VGSCs.

Steady-state inactivation recordings of tetrodotoxin-sensitive VGSCs were obtained by using a standard two-pulse protocol described in Fig. 5. Peak Na^+ currents recorded during the test pulse at the prepulse potential from -130 to -30 mV were normalized with respect to the maximal

value and plotted against the conditioning prepulse potential. Fig. 5 shows that both hainantoxin-III (1 nM) and hainantoxin-IV (50 nM) caused the half-maximal inactivation potential of Na^+ channels to shift approximate 10 mV in the hyperpolarizing direction without changing the slope factor (k) significantly (Fig. 5B).

3.3. Effects of toxins on the repriming kinetics of tetrodotoxin-sensitive Na^+ channels

The recovery kinetics from inactivation were observed by using a two-pulse protocol in which the second test pulse was triggered after a repolarizing interpulse to -80 mV lasting from 0.5 ms to 1 s. Under control conditions, almost no currents were induced by the second test pulse at 0.5 -ms interpulse time. At 50 – 100 -ms interpulse time, currents induced by second test pulse (I_{test}) were as large as that induced by first prepulse (I_{prep}) (Fig. 6A). Recovery from

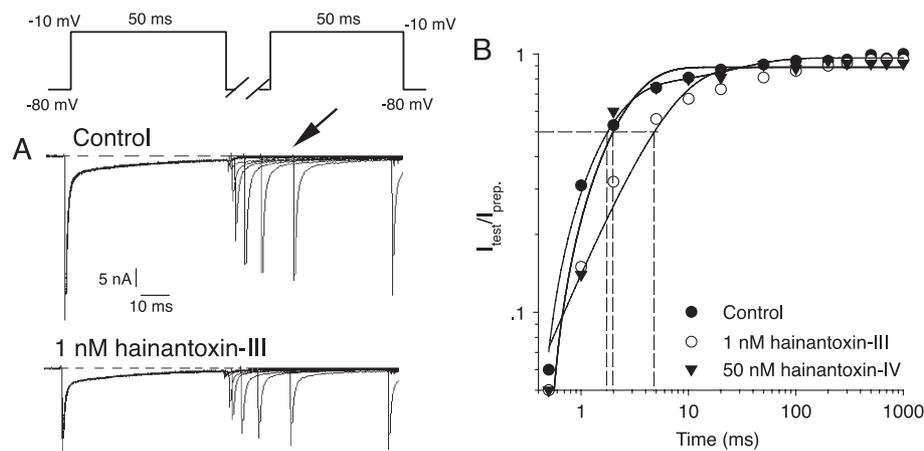


Fig. 6. Effects of two hainantoxins on the reprime kinetics of tetrodotoxin-sensitive Na^+ channels on rat dorsal root ganglion neurons. A dual-pulse voltage-clamp protocol was detailed in the inset using two 50-ms pulses from -80 to -10 mV with a varying interval from 0.5 ms to 1 s. (A) Typical current traces represent the recovery from inactivation in the absence (top) and presence (bottom) of 1 nM hainantoxin-III. (B) Data points showing the ratio of I_{test}/I_{prep} versus recovery time have been fitted using a dual-exponential equation. \bullet , Control; \circ , 1 nM hainantoxin-III; \blacktriangledown , 50 nM hainantoxin-IV.

inactivation was estimated by the ratio of I_{test} to I_{prep} . It was seen that hainantoxin-III slowed down channel recovery from inactivation significantly, and in the presence of hainantoxin-III (1 nM), the number of inactivated Na^+ channels is twice that under control condition at an interpulse interval of 1–4 ms. The interpulse interval at which half the maximal current has been recovered was increased by 1 nM hainantoxin-III from 2.3 to 4.8 ms (Fig. 6B). In contrast, hainantoxin-IV had no significant effect on the repriming kinetics (Fig. 6B).

3.4. Effects of toxins on voltage-gated Ca^{2+} currents

Two main categories of voltage-gated Ca^{2+} channel (VGCC) are expressed in dorsal root ganglion neurons: high voltage-activated (HVA) channels and low voltage-activated (LVA) channels. HVA and LVA channels can of course also be discriminated by their voltage-dependence and kinetics. Neither hainantoxin-III nor hainantoxin-IV affected VGCCs.

4. Discussion

In the present study, we characterized the actions of hainantoxin-III and hainantoxin-IV, isolated from the venom of the Chinese bird spider *S. hainana*, on ionic channels. Both toxins show no effect on Ca^{2+} channels and tetrodotoxin-resistant VGSCs. Hainantoxin-III and hainantoxin-IV are the main components of the crude venom and previous experiments demonstrated that the crude venom of the spider did not affect the rectifier-delayed potassium channels (Xiao et al., 2001). Therefore, it is reasonable to infer that hainantoxin-III and hainantoxin-IV fail to target rectifier-delayed potassium channels. Their actions on tetrodotoxin-sensitive Na^+ channels in dorsal root ganglion neurons are as follows: reducing the peak current amplitude; no shift in both initial activation and reversal potential but a significant shift in steady-state inactivation about 10 mV in the hyperpolarizing direction; only hainantoxin-III decreases the recovery rate from inactivation.

Spider venoms contain many peptide toxins and polyamide toxins. In general, the former are more specific for targeting voltage-gated channels than the latter. Peptide spider toxins can selectively affect VGSCs (δ -aractoxins (Nicholson et al., 1998)), Ca^{2+} channels (huwentoxin-I (Peng et al., 2001)) and ω -agatoxin IIIA (Mintz et al., 1991)) or potassium channels (HaTx1-2 and PaTx1-2 (Sanguinetti et al., 1997)). However, polyamide toxins (Argio-toxin-636; Scott et al., 1998) modulate the activities of several kinds of voltage-gated channels or/and receptor channels. Hainantoxin-III and hainantoxin-IV are specific to tetrodotoxin-sensitive VGSCs and it seems that their specificities are even higher than that of δ -aractoxin-Ar1 targeting tetrodotoxin-sensitive VGSC isoforms on both vertebrate and insect (Nicholson et al., 1998; Grolleau et

al., 2001), because these two peptides have no effect on cockroaches, the contractions of mouse diaphragm induced by direct electrical stimulus, or the twitch tension of frog heart (data not shown). Therefore, it is very likely that they target selectively the isoforms of VGSCs on mammal neuron membranes.

There are more than six neuronal receptor sites on Na^+ channels, which can be modulated by native toxins. To date, nearly 20 peptide toxins interacting with VGSCs have been fractionated from the spider venoms. Except huwentoxin-IV, other spider toxins, such as δ -aractoxins, μ -agatoxins and ω -palutoxins, all share a common mode of slowing the inactivation kinetics of currents, similar to scorpion α -toxins, or sea anemone toxins targeting channel site 3 (Escoubas et al., 2000). Huwentoxin-IV is a site 1-like toxin found first among the spider toxins (Peng et al., 2002). The most intriguing finding of the present study is that similar to huwentoxin-IV, hainantoxin-III and hainantoxin-IV show no effect on either activation or inactivation kinetics of VGSCs. The same results are also obtained on NG108-15 cells (unpublished result). Furthermore, the result is consistent with that of the crude spider venom, which does not slow the inactivation kinetics of Na^+ currents on NG108-15 cells (Xiao et al., 2001). Site 3 toxins induce the release of transmitters on the presynaptic membrane so that they cause spontaneous contractions of isolated rat vas deferens smooth muscle (Escoubas et al., 2000) and even increase the isolated diaphragm muscle twitch tension (Araujo et al., 1993). In contrast, hainantoxin-III and hainantoxin-IV blocked neuromuscular transmission in the isolated nerve-synapse preparations of rat vas deferens and mouse phrenic nerve-diaphragm in our previous studies. Furthermore, like tetrodotoxin, hainantoxin-III and hainantoxin-IV blocked the slowing currents induced by site 3 toxins completely. On the other hand, neither of the two toxins shifted the voltage-dependence of inactivation of VGSCs. Thus, it is highly unlikely that two hainantoxins target one of sites 2–6 on Na^+ channels. We are convinced that like huwentoxin-IV, two hainantoxins belong to the family of site 1 toxins.

Most of spider toxins affecting VGSCs can shift the steady-state inactivation of channels and accelerate the recovery rate of transition between the inactivated and resting states of the channel (Nicholson et al., 1998). Huwentoxin-IV is the spider toxin found first not to target site 3 on Na^+ channels. But it is unclear about the effects of huwentoxin-IV on steady-state inactivation and reprime kinetics of VGSCs. Both of two hainantoxins also shift the steady-state inactivation, and it seems that the inhibiting effects of tetrodotoxin-sensitive Na^+ current are not mainly due to the reduction in the availability of channels at resting potential, for that after toxin treatments, the amplitude of currents recorded from the holding potential of even -130 mV did not recover completely to the control size (see Fig. 6). However, the opposite result occurs in the presence of other spider toxins such as δ -aractoxins. The two hainantoxins affect the reprime kinetics

differently. A significant decrease in the rate of recovery from Na⁺ channel inactivation was observed. The similar effect of some antihyperalgesic agents (tricyclic antidepressant, 4030w92 (2,4-diarmino-5-5-(2,3-dichlorophenyl)-6-fluoromethylpyrimidine)) is reported recently on neuronal Na⁺ channel isoforms in bovine adrenal chromaffin cells (Pancrzo et al., 1998). Surprisingly, hainantoxin-IV unchanged the repriming kinetics of VGSCs. It indicates that two hainantoxins have a different action of S4 segments (voltage sensor) on channels.

It is not surprised that the toxicity of hainantoxin-IV is the same as that of huwentoxin-IV with the IC₅₀ value of 30 nM. Huwentoxin-IV is a typical inhibitor cystine knot (ICK) motif and by analyzing its three-dimensional structure, Peng et al. (2002) suggest residue Arg26 must be crucial to its binding to neuronal tetrodotoxin-sensitive VGSCs. Although huwentoxin-IV and hainantoxin-IV are isolated from the different spider venoms, they share 80% sequence identity with each other and the Arg26 is also conserved in hainantoxin-IV. It is interesting to note that hainantoxin-III is 40 times more potent than that of hainantoxin-IV and huwentoxin-IV. Although recent work indicates that hainantoxin-III also adopts an ICK motif (unpublished result), it shares limited sequence identity with huwentoxin-IV and hainantoxin-IV (Fig. 1). The Arg26 in huwentoxin-IV disappears in hainantoxin-III at the corresponding position. Because hainantoxin-III shares limited sequence identity with other peptides, it is difficult to determine the key residues of hainantoxin-III. However, we are convinced that like tetrodotoxin and saxitoxin, three spider toxins cannot target the same active residues on VGSC proteins. The two hainantoxins will be a useful tool to discriminate different Na⁺ channel subtypes and disclose the structure–function relationship of Na⁺ channels.

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