生物化学与生物物理学报
ISSN 0582-9879 ACTA BIOCHIMICA et BIOPHYSICA SINICA 2003, 35(1): 82 - 85 CN 31-1300/Q

# Inhibition of Sodium Channels in Rat Dorsal Root Ganglion Neurons by Hainantoxin-IV, a Novel Spider Toxin

XIAO Yu-Cheng, LIANG Song-Ping *

( *College of Life Sciences*, *Hunan Normal University*, *Changsha* 410081, *China* )

**Abstract** The effects of Hainantoxin-IV (HNTX-IV), a neurotoxic peptide isolated from the venom of the Chinese bird spider *Seleconosmia hainana*, on the adult rat dorsal root ganglion (DRG) neurons were investigated. Using the whole-cell patch-clamp technique HNTX-IV inhibited mammal neural TTX-sensitive (TTX-S) sodium currents evidently but the toxin failed to affect TTX-resistant (TTX-R) ones. The inhibition of HNTX-IV is dose-dependent with the $IC_{50}$ value of 44.6 nmol/L. The toxin didn't affect the activation and inactivation kinetics of sodium currents, but it caused a 10.1 mV hyperpolarizing shift in the voltage midpoint of steady-state sodium channel inactivation on DRG neurons. The results indicated that HNTX-IV, a novel spider toxin, maybe alternate voltage-gated sodium channels through a mechanism distinct from other spider toxins such as δ-ACTXs, μ-agatoxins I-VI which targeted the receptor site 3 to slow the inactivation kinetics of sodium currents.

**Key words** spider toxin; dorsal ganglion neurons; sodium current; whole-cell patch-clamp

The venoms of many animals such as snake, scorpion, marine snail and spider contain many different kinds of toxins which can affect sodium channels with different mechanism. These natural toxins, especially tetrodotoxin (TTX) and scorpion α, β-toxins, are important useful tools for studying the structure-function relationship of sodium channels. At least six sites (1 - 6) on neural sodium channels have been disclosed by using these toxins[1]. About twenty spider toxins are found to alternate sodium channels, such as δ-ACTXs[2], μ-agatoxins I-VI[3], PhTx2[4]. These spider toxins are peptides consist of 40 - 80 residues with four disulfide bonds. They can slow the inactivation kinetics of currents in a similar manner to α-scorpion toxin by banding the site 3 on sodium channels[2]. Hainantoxin-IV (HNTX-IV) is a spider toxin isolated from the venom of the Chinese bird spider *Seleconosmia hainana*[5]. The sequence of HNTX-IV have been determined to be: $NH_2$-ECLGFGKGCNPSNDQCCKSSNLVCSRKHRWCKYEI-$CONH_2$ with three disulfide bonds. Its chemical synthesis has been achieved by a solid-phase method[6]. The intraperitoneal $LD_{50}$ values of the toxin in mice is 0.2 mg/kg. It can block neuromuscular transmission in the isolated nerve-synapse preparations of rat vas deferens and mouse phrenic nerve-diaphragm. Here we report the effects of HNTX-IV on voltage-gated sodium currents in adult rat dorsal root ganglion (DRG) cells.

Received: July 23, 2002 Accepted: September 13, 2002

This work was supported by a grant from the National Natural Science Foundation of China (No.30170193)

* Corresponding author: Tel, 86-731-8872556; Fax, 86-731-8861304; e-mail, liangsp@public.cs.hn.cn

## 1 Materials and Methods

### 1.1 Purification of toxin

HNTX-IV was purified using reverse phase HPLC followed by ion-exchange chromatograph as described earlier[5].

### 1.2 Cell isolation procedures

Rat DRG neurons were acutely dissociated and maintained in a short-term primary culture using the method described by Wang *et al*.[7]. Briefly, 30-day adult Sprague-Dawley rats of either sex were killed by decapitation and 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, Sigma), collagenase (1.0 g/L, type IA, Sigma) and DNase (0.1 g/L, type III, Sigma) to incubate at 34 ℃ for 30 min. Trypsin inhibitor (1.5 g/L, type II-S, Sigma) was used to terminate enzyme treatment. After transferred into 35 mm culture dishes (corning, Sigma), the DRG cells were incubated in $CO_2$ incubator (5% $CO_2$, 95% air, 37 ℃) for 1 - 4 h before patch-clamp experiment.

### 1.3 Electrophysiological recordings

Sodium 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 ℃). 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 filled with internal solution contained (mmol/L): CsF 135, NaCl 10, HEPES 5, with pH adjusted to 7.0 with 1 mol/L CsOH. The external bathing solution contained (mmol/L): NaCl 30, CsCl 5, *D*-glucose 25, $MgCl_2$ 1, $CaCl_2$ 1.8, HEPES 5, tetraethylammonium (TEA) chloride 20, tetramethylammonium chloride 70, with pH adjusted to 7.40 with 1 mol/L TEA hydroxide[2]. The osmolarities of both internal solution and external solution were adjusted to 290 mOsmol with sucrose. An Ag-AgCl pipette/150 mmol/L 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, experiments didn't commence for a period of more than 3 – 4 min to allow adequate equilibration between the micropipette solution and the cell interior. Drug-containing solutions of about 10 μL volume were applied by pressure injection with a microinjector (IM-5B, Narishige). All chemical reagents were purchased from Sigma.

## 2 Results

### 2.1 Effects of HNTX-IV on voltage-gated sodium currents

The DRG cells with diameters of 20 – 40 μm were selected for experiments, for larger DRG cells from older animals tend to express fast TTX-S sodium currents while smaller ones (10 – 20 μm) tended to express slow TTX-resistant (TTX-R) sodium currents[8]. TTX (200 nmol/L) was added into external solution to separate TTX-R sodium currents from TTX-S ones. Under voltage-clamp conditions, sodium currents on DRG cells were elicited by 50 ms depolarization to – 10 mV from a holding potential of – 80 mV every 1 min. Fig. 1(A) showed that HNTX-IV reduced the peak amplitude of TTX-S sodium currents to a maximum effect within 3 min on DRG neurons. 10 and 50 nmol/L HNTX-IV could reduce control currents amplitude by (28.8 ± 6.5)% and (57.2 ± 5.4)% ($\bar{x} \pm s$), respectively. The reductions of HNTX-IV were concentration-dependent with the $IC_{50}$ value of 44.6 nmol/L [Fig. 1(C)]. After reduction, the shape of sodium currents was similar to that of control, indicating that HNTX-IV didn't change the activation and inactivation kinetics of TTX-S sodium currents. In contrary, TTX-R sodium currents weren't inhibited significant on DRG cells by 50 nmol/L HNTX-IV [Fig. 1 (B)], implying that HNTX-IV didn't alternate the steady-state activation and inactivation kinetics of TTX-R sodium channels.

### 2.2 Effects of HNTX-IV on the current-voltage relationship of TTX-sensitive sodium channel

The current-voltage curve of TTX-S sodium channels were obtained by depolarization steps from a holding potential of – 80 mV to + 50 mV. Under control conditions TTX-S sodium currents were initial elicited at the – 50 mV and reached maximal amplitude at around – 20 mV. After 50 nmol/L HNTX-IV treatment for 3 min, both the threshold of activation and the active voltage of peak inward currents weren't changed, and the membrane reverse potential wasn't shifted evidently, too (Fig. 2).

### 2.3 Effects of HNTX-IV on inactivation kinetics of TTX-S sodium channels

Using a standard two-pulse protocol, we

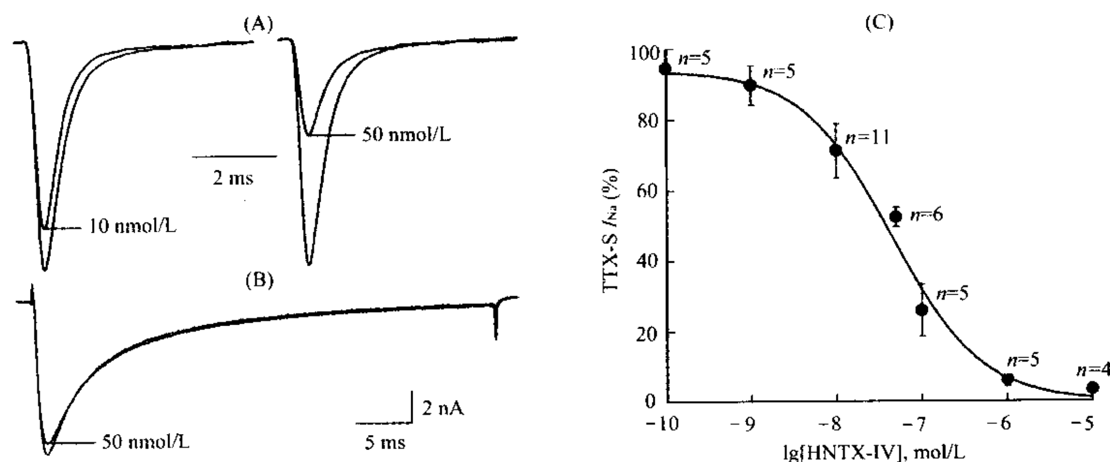


**Fig.1 Effects of HNTX-IV on the TTX-S (A) and TTX-R sodium currents (B) and the dose-dependent block of TTX-S sodium currents (C)**

Currents were induced by a 50 ms depolarizing potential of – 10 mV from a holding potential of – 80 mV. In (C) data points ($\bar{x} \pm s$) were fitted according to Boltzmann equation.

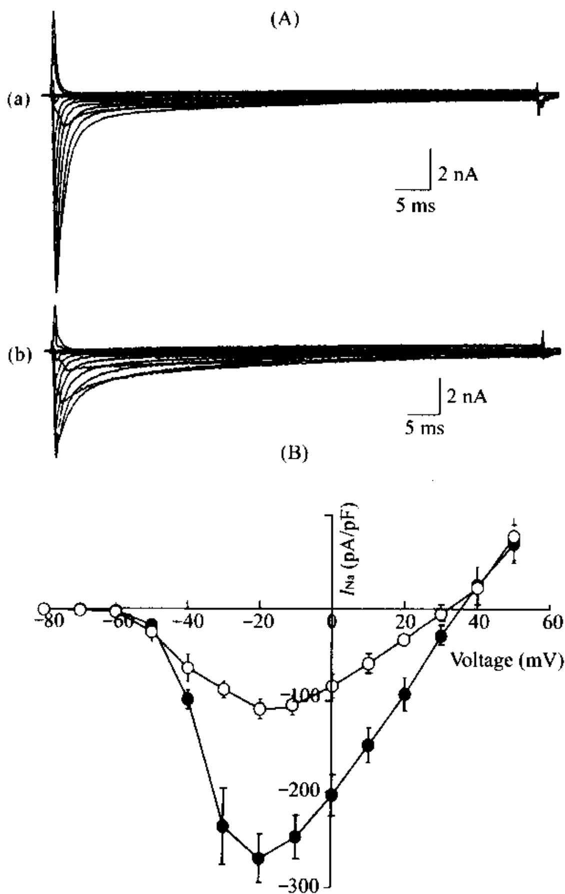


**Fig.2 Effects of HNTX-IV on rat DRG peak sodium currents ($n = 5$)**

(A) Currents were evoked before (a) and after (b) application of HNTX-IV (50 nmol/L) by 50 ms depolarizing steps from − 80 mV to + 50 mV in + 10 mV increments from the holding potential of − 80 mV. (B) The relationship of voltage and sodium currents in the presence and absent of HNTX-IV. ●, control ; ○, 50 nmol/L HNTX-IV.

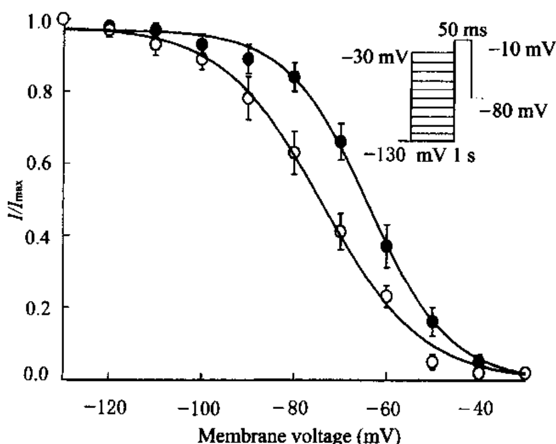


**Fig.3 Effects of HNTX-IV on the steady-state inactivation of TTX-S sodium channels in rat DRG neurons ($n = 5$)**

The curves were fitted by the Boltzmann equation : $I/I_{max} = 1/\{1 + \exp[(V - V_{1/2})/k]\}$ where $V$ is holding voltage, $V_{1/2}$ is the voltage at which $I$ is 0.5 $I_{max}$, and $k$ is the slop factor. ●, control ; ○, 50 nmol/L HNTX-IV.

quantified the changes of the voltage dependence of steady-state sodium channel inactivation produced by HNTX-IV. Under whole-cell patch clamp condition, DRG cells were held at a prepulse voltage ranged from − 130 mV to − 30 mV in + 10 mV increments for 1 s. After 0.5 ms interpulse interval a test pulse to − 10 mV for 50 ms was delivered to evoke TTX-sensitive sodium currents. Peak sodium currents recorded during the test pulse were normalized to the maximal value and plotted against the conditioning prepulse potential. Fig. 3 showed that 50 nmol/L HNTX-IV caused the half-maximal inactivation potential of sodium channels to shift approximate 10.1 mV in hyperpolarizating direction from (− 64.0 ± 1.1) mV to (− 74.1 ± 1.6) mV ($\bar{x} \pm s$, $n = 4$) and the slop factor ($k$) was increased by 2.0 mV from (8.8 ± 0.5) mV of control to (10.8 ± 0.8) mV.

## 3 Discussion

The results of previous experiment in our laboratory showed that the contractions of mouse diaphragm induced by direct electrical stimulus weren't affected by HNTX-IV, and no visible symptoms were observed after injection of HNTX-IV into cockroaches (inacimal dose 60 μg/g body weight)[6], which suggests that HNTX-IV should target selectively the sodium channel isoforms on mammal neuron membranes. Although there are two kinds of sodium channels (TTX-S and TTX-R) on neurons, HNTX-IV inhibited the former selectively similar to other spider toxins such as δ-ACTXs[2]. Nanomolar concentration of the toxin evidently inhibited sodium currents induced on adult rat DRG neurons. HNTX-IV didn't shift the membrane reverse potential of sodium channels on DRG neurons, implying that it didn't change the ion selectivity of channels. The most intriguing finding of the present study, however, was that the spider toxin didn't affect the inactivation kinetics of TTX-S sodium currents on DRG neurons. The similar result was also obtained on NG108-15 cells (unpublished result). Furthermore, the result was consistent with that of the crude spider venom which didn't slow the inactivation kinetics of sodium currents on NG108-15 cells[9]. To date almost twenty spider toxins are found to affect sodium channels, but they all have a common mode of action, similar to that of α-scorpion toxin and sea anemone toxins by banding the site 3 on sodium channels. They slow down the inactivation kinetics of currents to prolong the time course of action potential, so that they cause spontaneous contractions of isolated rat vas deferens smooth muscle[2] and even increase the isolated diaphragm muscle twitch tension[4]. In contrary, HNTX-IV didn't show such properties but blocked neuromuscular transmission in the isolated nerve-synapse preparations of rat vas deferens and mouse phrenic nerve-diaphragm in our previous studies[6]. So, HNTX-IV may be a novel spider toxin which alternates voltage-gated sodium

channels through a mechanism distinct from other spider toxins.

**Acknowlegements** We are grateful to Prof. LI Zhi-Wang at Tongji Medical University for technical assistance in DRG cell isolation.

## References


1 Cestele S, Ben Khalifa RB, Pelhate M, Rochat H, Gordon D. α-Scopion toxins binding on rat brain and insect sodium channels reveal divergent allosteric modulations by brevetoxin and veratridine. *J Biol Chem*, 1995, **270**(25): 15153–15161

2 Szeto TH, Birinyi-Strachan LC, Smith R, Connor M, Christie MJ, King GF, Nicholson GM. Isolation and pharmacological characterization of δ-atracotoxin-Hv1b, a vertebrate-selective sodium channel toxin. *FEBS Lett*, 2000, **470**(3): 293–299

3 Skinner WS, Adams ME, Quistad GB, Kataoka H, Cesarin BJ, Enderlin FE, Schooley DA. Purification and characterization of two classes of neurotoxins from the funnel web spider, *Agelenopsis aperta*. *J Biol Chem*, 1989, **264**(4): 2150–2155

4 Araujo DA, Cordeiro MN, Diniz CR, Beirao PS. Effect of a toxic fraction, PhTx2, from the spider *Phoneutria nigriventer* on the sodium current. *Naunyn Schmiedebergs Arch Pharmacol*, 1993, **347**(2): 205–208

5 Liang SP, Peng XJ, Huang RH, Chen P. Biochemical identification of *Selenocosmia hainana sp. nov.* from south China [Araneae, Theraphosidae]. *Life Science Research*, 1999, **3**(4): 299–303
(引自:生命科学研究)

6 Liu ZH, Chen P, Liang SP. Synthesis and oxidative refolding of Hainantoxin-IV. *Acta Biochim Biophys Sin*, 2002, **34**(4): 516–519
(引自:生物化学与生物物理学报)

7 Wang QW, Li Q, Li ZW. Inhibitory effects of clonidine on GABA-activated currents in DRG neurons. *Acta Physiol Sin*, 1998, **50**(1): 19–27
(引自:生理学报)

8 Su X, Wachtel RE, Gebhart GF. Capsaicin sensitivity and voltage-gated sodium currents in colon sensory neurons from rat dorsal root ganglia. *Am J Physiol*, 1999, **277**(6 Pt 1): G1180–G1188

9 Xiao YC, Peng K, Chen XD, Liang SP. Inhibition of voltage-gated sodium channels in NG108-15 by the venoms from three kinds of spider. *Life Science Research*, 2001, **5**(5): 42–47
(引自:生命科学研究)


# 海南捕鸟蛛毒素-IV对大鼠背根神经节细胞钠通道的抑制影响

**肖玉成　梁宋平*

(湖南师范大学生命科学学院,长沙 410081)**

**摘要**　海南捕鸟蛛毒素-IV(HNTX-IV)是从中国捕鸟蛛 *Seleconosmia hainana* 粗毒中分离得到的一种肽类神经毒素,在成年大鼠背根神经节(DRG)细胞上观察了该毒素对电压门控钠通道的影响。在全细胞膜片钳条件下,HNTX-IV能明显抑制哺乳动物神经性河豚毒敏感型(TTX-S)钠电流,但不影响河豚毒不敏感型(TTX-R)钠电流。HNTX-IV对DRG细胞TTX-S钠电流的抑制作用具有浓度依从性,其有效半抑制浓度($IC_{50}$)为44.6 nmol/L。该毒素不影响DRG钠电流的激活与失活时间特征,但能导致钠通道的半数稳态失活电压向超极化方向漂移约10.1 mV。结果表明HNTX-IV是一种新型的蜘蛛毒素,其影响电压门控钠通道的机制可能有别于那些结合于通道位点3来延缓钠电流失活时间特征的蜘蛛毒素如δ-澳洲漏斗网蛛毒素、μ-美洲漏斗网蛛毒素I-VI等。

**关键词**　蜘蛛毒素;背根神经节;钠电流;全细胞膜片钳

**收稿日期**:2002-07-23　**接受日期**:2002-09-13
**国家自然科学基金重大项目资助**(No.30170193)
* **联系人**:Tel,0731-8872556;Fax,0731-8861304;e-mail,liangsp@public.cs.hn.cn