



# The presynaptic activity of huwentoxin-I, a neurotoxin from the venom of the Chinese bird spider *Selenocosmia huwena*

Song-Ping Liang\*, Xiang-Ding Chen, Qin Shu,  
Yongqun Zhang, Kuan Peng

*Department of Biology, Hunan Normal University, Changsha 410081, People's Republic of China*

Received 24 May 1999; accepted 18 October 1999

---

## Abstract

Three different types of isolated nerve–synapse preparations, guinea pig ileum, rat vas deferens and toad heart, were used to investigate the physiological activity of Huwentoxin-I, a neurotoxin from the venom of the spider *Selenocosmia huwena*. The twitch response of isolated guinea pig ileum induced by electrical stimulus can be inhibited by HWTX-I. After blockage, contraction of the ileum can be induced by exogenously applied acetylcholine. HWTX-I caused the inhibition of the twitch response to electrical nerve stimulation in the rat vas deferens. After the twitch was completely inhibited, noradrenaline triggered rhythmic contraction of the vas deferens. The inhibitory effect on heart of toad induced by stimulating sympathetic-vagus nerve can be reversed by HWTX-I, although exogenously applied acetylcholine still acts as an effective inhibitor. All of these results support the conclusion that HWTX-I has the presynaptic activity that effects the release of neurotransmitter from the nerve endings of both the cholinergic synapse and the adrenergic synapse. © 2000 Elsevier Science Ltd. All rights reserved.

---

## 1. Introduction

Huwentoxin-I (HWTX-I), a neurotoxic peptide isolated from the venom of the

---

\* Corresponding author. Tel.: +86-731-887-2556; fax: +86-731-886-1304.  
E-mail address: liangsp@public.cs.hn.cn (S.-P. Liang).

Chinese bird spider *Selenocosmia huwena*, can reversibly block neuromuscular transmission in an isolated mouse phrenic nerve-diaphragm preparation. (Liang et al., 1993). The intraperitoneal and intracisternal LD<sub>50</sub> values of the toxin in mice were 0.70 mg/kg and 9.40 µg/kg, respectively. The amino acid sequence and the location of the three disulfide bonds have also been determined (Zhang and Liang, 1993) as showed in Fig. 1. The three-dimensional structure in aqueous solution of native huwentoxin-I has been determined by two-dimensional <sup>1</sup>H NMR (Qu et al., 1995, 1997). The molecule has a compact structure consisting of a small triple-stranded antiparallel β-sheet and a knot of three disulfide bonds (Fig. 1), a structure which has been adopted by several other small proteins, such as ω-conotoxin, squash trypsin inhibitor and gurmardin. (Holak et al., 1991; Pallaghy et al., 1993, 1994; Arai et al., 1995). Zhou et al. (1997) suggested that huwentoxin-I blocks postsynaptic ACh receptors based on the result that D-tubocurarine and huwentoxin-I showed competitive effects in the blockade of neuromuscular

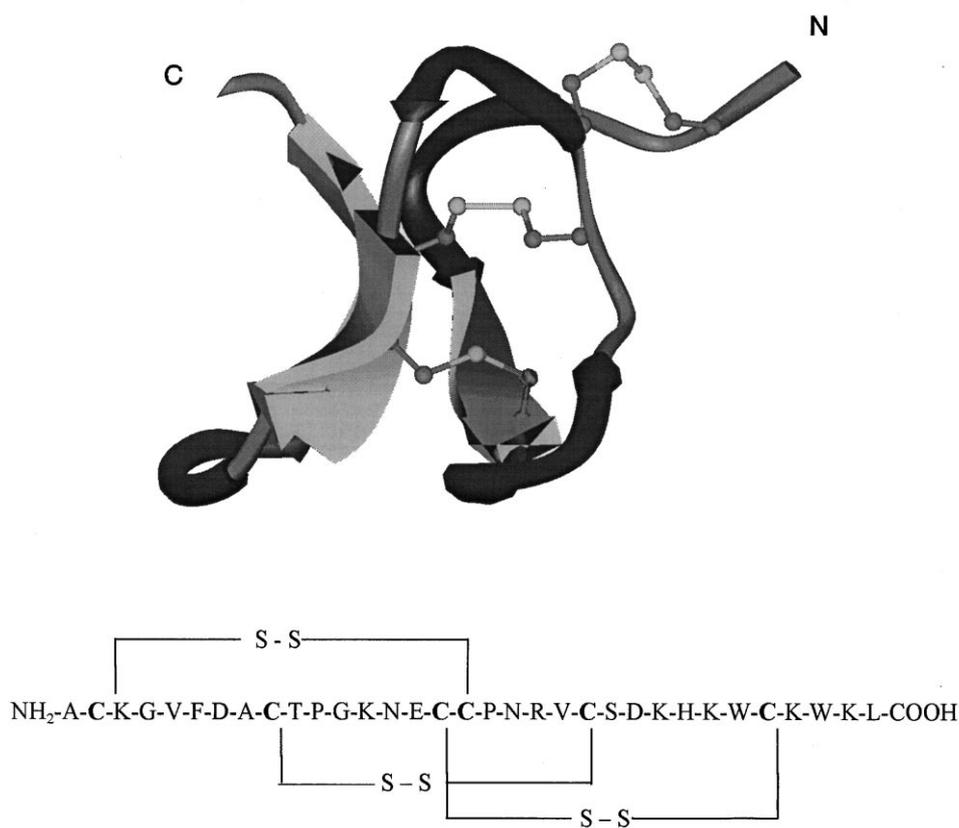


Fig. 1. Polypeptide backbone folding seen in the three-dimensional structure (top) and the amino acid sequence and the disulfide bond arrangement (bottom) of huwentoxin-I.

transmission and that HWTX-I can reduce the probability of ACh-induced channel activity on *Xenopus* embryonic myocytes. However, recent experiments in our laboratory on the binding of the radioisotope labeled HWTX-I to the ACh receptor of the Torpedo electric organ gave completely negative results. We also have observed no effect of HWTX-I on the nicotinic ACh receptor in the TE671 human muscle cell line. These results are suggestive of the complicated nature of the physiological activity of HWTX-I. In order to investigate further the mechanism of the blockade of neuromuscular transmission by this toxin, we conducted, as described in the present work, three electro-physiological experiments: (1) the effect of HWTX-I on the contraction of isolated guinea pig ileum induced by electrical stimulus, (2) the effect of HWTX-I in transmitter release from sympathetic neurons in the isolated vas deferens of rats and (3) the effect of HWTX-I to the inhibition on contraction of toad heart induced by stimulating the sympathetic-vagus nerve. In all the experiments we obtained data that suggest that HWTX-I acts on the peripheral nervous system to inhibit transmitter release from nerve terminals.

## 2. Materials and methods

### 2.1. Animals and chemicals

Adult guinea pigs (350–500 g) and adult male Sprague-Dawley rats (250–300 g) were from Hunan Medical University. Adult toads were collected from a local farm. Huwentoxin-I was isolated from the venom of the female spider (*S. huwena*) and purified by means of ion-exchange and RP-HPLC as described in our previous paper (Liang et al., 1993). Acetylcholine (ACh), adrenaline, atropine and phentolamine were from Sigma. Noradrenaline bitartrate was from Wuhan Pharmaceutical (Wuhan, China).

### 2.2. Isolated guinea pig ileum

The method used was basically according to the procedure of Harry (1964). Segments 4 cm in length were taken from the ileum of a guinea pig 15 cm from the ileo-caecal junction. A segment was placed into a 10-ml water-jacketed glass bath (32°C) containing Tyrode's solution (NaCl 136.7 mM; KCl 2.7 mM; CaCl<sub>2</sub> 1.82 mM; NaHCO<sub>3</sub> 1.19 mM; MgCl<sub>2</sub> 1.05 mM; NaH<sub>2</sub>PO<sub>4</sub> 0.41 mM; glucose 5.6 mM) through which was bubbled 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Rectangular current pulses of 0.05 ms duration with a strength of 25 V were applied to the platinum electrodes; the intraluminal electrode was made the anode. The contraction of the gut was recorded with a Chengdu Instruments model LMS-2B two-channel physiology recorder. Prior to the start of the experiment, the isolated guinea pig ileums were equilibrated in Tyrode's solution for 30 min. The effect of the HWTX-I was tested by adding it to the bath with the final concentration of  $1.3 \times 10^{-6}$  M in Tyrode's solution.

### 2.3. Isolated working toad heart

The method for the preparation of the isolated working toad heart and the perfusion system were as described by Guo et al. (1989). The hearts were removed from the body with all the pericardiac arteriae and veins tied up separately and was mounted on a working heart apparatus. The vagosympathetic nerve trunk of one side was isolated and attached to the heart without loss of efficacy. Most of the ventricle was cut away and the atrial septum was removed, and then a frog-heart tube was inserted into the auricle. The junction of the auricle and the tube was seamed and 0.3-ml Ringer's solution (NaCl 102.6 mM; KCl 1.01 mM; CaCl<sub>2</sub> 0.91 mM; NaHCO<sub>3</sub> 1.19 mM) was injected into the auricle. A Chengdu Instrument model JJC-3 stimulator with two electrodes placed on the vagosympathetic nerve trunk stimulated the auricle with frequency of 10–20 Hz and strength of 5–20 V. The contractions of the isolated hearts were recorded by a set of model LMS-2B two-channel recorder.

### 2.4. Vas deferens of rats

The method of Lew et al. (1997) was used. Adult male Sprague Dawley rats were killed by CO<sub>2</sub> anesthesia followed by decapitation. Vasa deferentia were mounted in 5-ml organ baths with the top of each tissue attached to an isometric force transducer and the bottom attached to a movable support and straddled with platinum stimulating electrodes. The vasa were immersed in Krebs solution (NaCl 117.3 mM; KCl 4.7 mM; CaCl<sub>2</sub> 2.55 mM; NaHCO<sub>3</sub> 2.5 mM; MgCl<sub>2</sub> 1.46 mM; NaH<sub>2</sub>PO<sub>4</sub> 1.17 mM) gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub> and were stretched by a passive force about 10 mN. After an equilibration period of 30 min with frequent

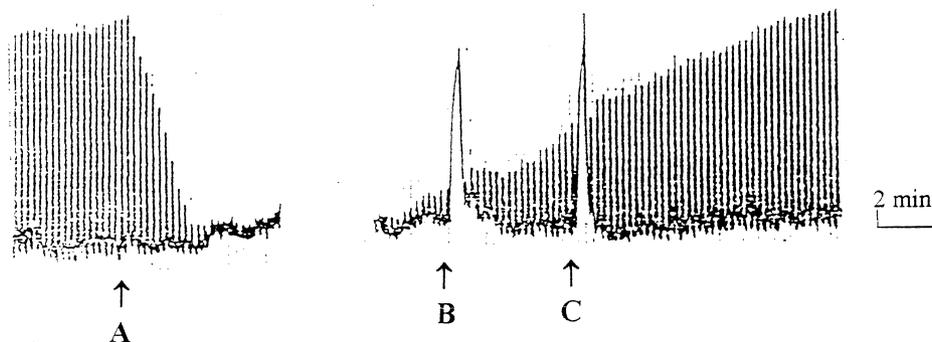


Fig. 2. The inhibitory effect of HWTX-I on the twitch response of guinea pig ileum by electrical stimulus. Stimulation conditions: Rectangular current pulses of width 0.05 ms, strength 25 V and frequency 30/min at 32°C. (A) HWTX-I ( $1 \times 10^{-6}$  M) (B, C) washes with Tyrode solution. (The two peaks after B and C were caused by the change of the Tyrode solution in the chamber and were not the twitch response of guinea pig ileum.)

changes of medium, the vasa were stimulated with single electrical field pulses (100 V, 0.14-ms duration) every 10 s. The resulting twitch responses were mediated by sympathetic nerves and were recorded on LMS-2B two-channel recorder.

### 3. Results

#### 3.1. Effect of HWTX-I on contraction of isolated guinea pig ileum

Electric stimulus can cause synchronous twitches of guinea pig ileum (Fig. 2). In the presence of HWTX-I at the concentration of  $8.8 \times 10^{-6}$  M, the twitches were totally inhibited within  $5.17 \pm 0.23$  min ( $n = 5$ ). After irrigating the ileum repeatedly with Tyrode's solution, the twitch response of the ileum to electrical stimulus was recovered finally. This result indicates that the inhibition by HWTX-I is reversible. Fig. 3 shows that, in the absence of electrical stimulus, the addition of acetylcholine ( $3.7 \times 10^{-8}$  M) to the bath caused the contraction of guinea pig ileum. Nearly the same degree of contraction was caused by acetylcholine which was added to the bath after the twitch response had been completely inhibited by the HWTX-I. This result demonstrated that in the presence of HWTX-I and after inhibition had taken place the ileum was still sensitive to acetylcholine. There is an evident difference between the effects of the atropin and HWTX-I on the twitches of the ileum. Fig. 3 shows that, after the twitch response was completely recovered by washing out the HWTX-I, the addition of atropin ( $1 \times 10^{-6}$  M) caused rapid inhibition of the twitch response and on this stage the addition of acetylcholine could not cause the contraction of the ileum. The recovery was also very slow during the irrigating the ileum with Tyrode's solution.

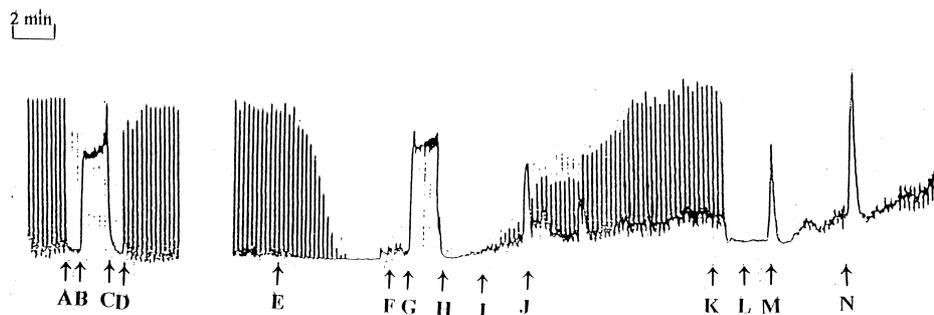


Fig. 3. The effect of HWTX-I on guinea pig ileum contraction induced by acetylcholine. stimulation conditions: Rectangular current pulses of width: 0.05 ms, strength 25 V and frequency 30/min at 32°C. (A, F), stimulus off; (B, G, L). Add ACh ( $3.7 \times 10^{-8}$  M); (C, H, J, M, N). Washing with tyrode solution; (D, I), stimulus on; (E) HWTX-I ( $1.3 \times 10^{-6}$  M) (K) add atropin ( $1.0 \times 10^{-6}$  M).

### 3.2. Effect of HWTX-I on the contraction of the isolated vas deferens

HWTX-I ( $1.3 \times 10^{-7}$ – $2.6 \times 10^{-6}$  M) caused a gradual concentration-related reduction in the size of the twitch response to electrical nerve stimulation in rat vas deferens. Fig. 4 shows that the twitch response was completely inhibited by HWTX-I ( $1.3 \times 10^{-6}$  M) within 30 min. After blockade and in the presence of HWTX-I, the addition of noradrenaline ( $5.5 \times 10^{-6}$  M) triggered rhythmic contraction of the vas deferens without electrical stimulation. These rhythmic contractions were completely abolished by the addition of phentolamine ( $6 \times 10^{-6}$  M). The twitch response of isolated vas deferens by electrical stimulation is due to the activation of the  $\alpha$  type receptor by adrenaline, which is released from endings of adrenergic nerve. This is demonstrated in our experiment by the effect of phentolamine, an inhibitor of the  $\alpha$  type receptors. Noradrenaline acts almost exclusively on  $\alpha$  receptor. The results of our experiment indicated that after the blockage of the twitches of the vas deferens by HWTX-I, noradrenaline still can cause the contraction of the vas deferens.

### 3.3. Effect of HWTX-I on toad heart contraction

The beating of the heart is controlled by a relatively complicated nerve system which is related to both the cholinergic and adrenergic synapses. The toad auricle has the natural rhythm of systole and diastol. When appropriate electronic stimulus was exerted on the vagosympathetic nerve trunk, the initial rhythm vanished gradually and the auricle stopped beating finally (Fig. 5). In the presence of HWTX-I ( $8.8 \times 10^{-6}$  M), the inhibitory effect of electronic stimulus was canceled within  $14.75 \pm 0.29$  min ( $n = 5$ ), while acetylcholine still acted as an effective inhibitor at this stage of time.

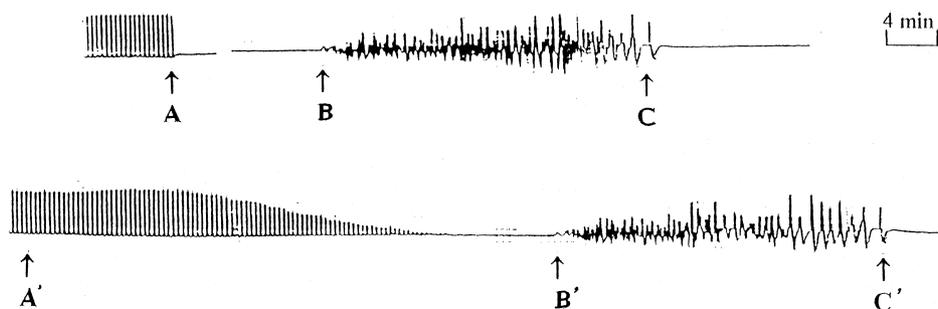


Fig. 4. The effect of HWTX-I on the twitch response of the isolated vas deferens by electrical stimulation. Stimulation condition: rectangular current pulses of width: 0.15 ms, strength 100 V and frequency 6/min at 32°C. Top (control): (A) stimulus off; (B) noradrenaline ( $3.5 \times 10^{-6}$  M); (C) phentolamine ( $6 \times 10^{-6}$  M). Bottom (effect of HWTX-I): (A') HWTX-I ( $1.3 \times 10^{-6}$  M); (B') noradrenaline ( $5.5 \times 10^{-6}$  M); (C') phentolamine ( $6 \times 10^{-6}$  M).

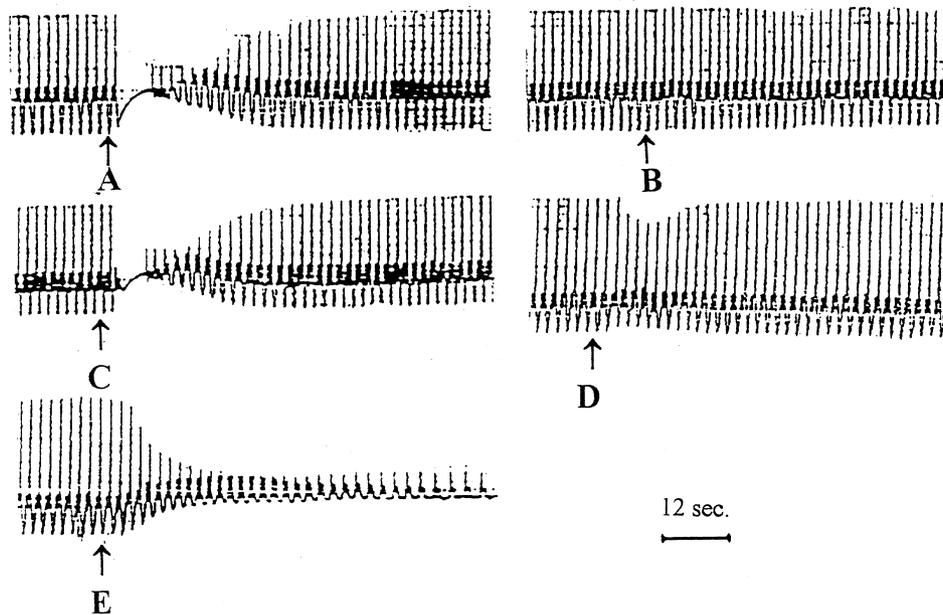


Fig. 5. The effect of HWTX-I on the response of isolated heart of toad by electrical stimulus. Stimulation condition: rectangular current pulses of width: 0.1 ms, strength 25 V and frequency 10/min. at 32°C. (A) Stimulus on; (B) add HWTX-I ( $8.8 \times 10^{-6}$  M); (C) Stimulus on 6.5 min after the addition of HWTX-I; (D) stimulus on 14 min after the addition of HWTX-I; (E) acetylcholine ( $1.2 \times 10^{-6}$  M).

After irrigating the auricle repeatedly with Ringer's solution, the inhibition of toad heart contraction induced by stimulating sympathetic-vagus nerve was recovered.

Fig. 6 shows that after atropinization ( $2.3 \times 10^{-4}$  M) of the toad auricle, electronic stimulus of the vagosympathetic nerve trunk caused the auricle to beat faster and stronger. In the presence of HWTX-I ( $8.8 \times 10^{-6}$  M), the strengthening effect on the beating of the auricle by electronic stimulus was canceled within  $2.67 \pm 0.27$  min ( $n = 5$ ) while adrenaline ( $3.7 \times 10^{-4}$  M) was still an effective cardio-activator at this stage of time. Irrigating the auricle with Ringer's solution could relieve it from HWTX-I blockade.

#### 4. Discussion

It is generally accepted that the twitch response of guinea pig ileum in the type of experiment described in this paper is due to the activation of the M type ACh receptor by acetylcholine, which is released by the stimulation on the

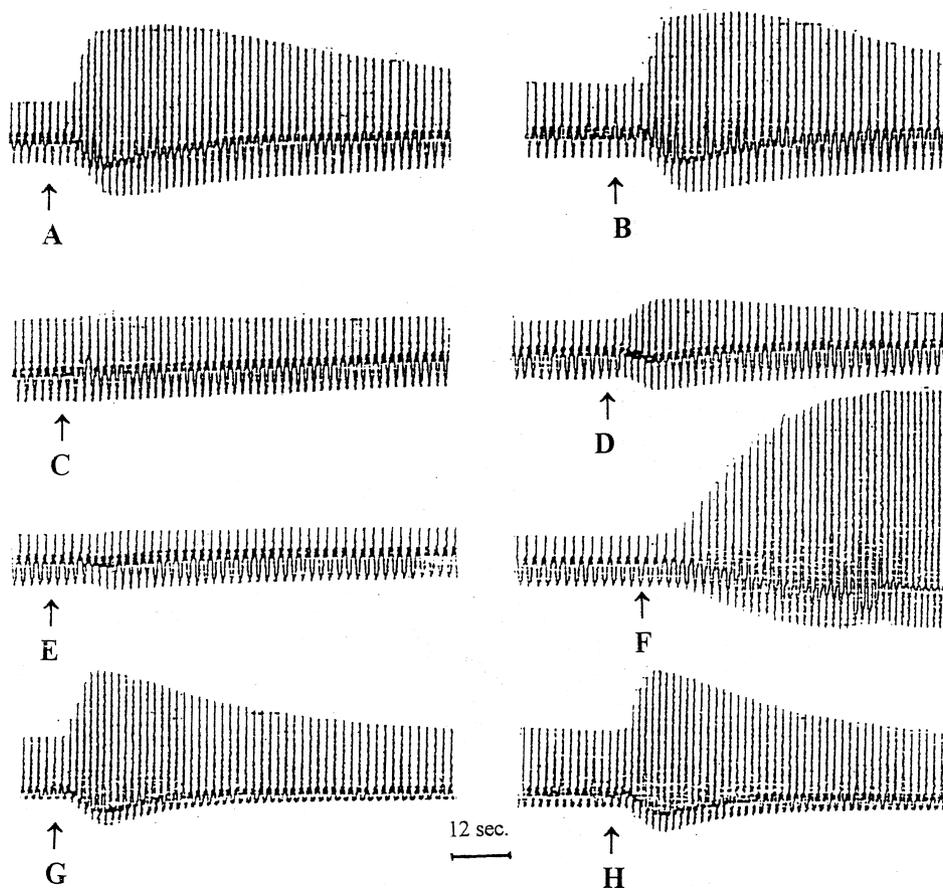


Fig. 6. The effect of HWTX-I on the response of the atropin treated isolated heart of toad by electrical stimulus. The toad auricle was first treated with atropin ( $2.3 \times 10^{-4}$  M). Stimulation condition: Rectangular current pulses of width: 0.1 ms, strength 25 V and frequency 10/min at  $32^{\circ}\text{C}$ . (A, B) stimulus on; (C) add HWTX-I ( $8.8 \times 10^{-6}$  M); (D) stimulus on 2.75 min after the addition of HWTX-I; (E) stimulus on, 6 min after the addition of HWTX-I; (F) adrenaline ( $1.8 \times 10^{-5}$  M), 8.5 min after the addition of HWTX-I; (G) stimulus on 12 min after irrigating the auricle with Ringer's solution. (H) Stimulus on 14.5 min after irrigating the auricle with Ringer's solution.

parasympathetic nerve. This was demonstrated in our experiment by the effect of atropine, an inhibitor of the M type ACh receptor. We have also determined that in the presence of phentolamine, an inhibitor of  $\alpha$ -type adrenaline receptors, the effect of HWTX-I on the twitch response of the ileum was not changed (data is not presented). All these results suggest that the inhibitory effect of HWTX-I on guinea pig ileum is due to the blockage of the release of ACh or to some process before the release of ACh.

The results of the experiment of the vas deferens indicated that after the

blockage of the twitches of the vas deferens by HWTX-I, noradrenaline still could cause the contraction of the vas deferens. Since noradrenaline acts almost exclusively on  $\alpha$  receptor, therefore the postsynaptic  $\alpha$  receptor is not the action site of HWTX-I. We suggest that blockade is due to the inhibition of on the release of adrenaline from adrenergic nerve ending.

Nearly the same conclusion can be drawn from the results of the experiment of toad heart. In the presence of atropine the postsynaptic M type Ach receptors were inhibited. The strengthening effect of the auricle is due to the release of adrenaline by adrenergic nerve ending, which is caused by the electronic stimulus of the vagosympathetic nerve trunk. This strengthening effect can be canceled by HWTX-I while the postsynaptic membrane was still sensitive to adrenaline. This result indicates that HWTX-I has no influence on the receptors of the postsynaptic membrane but does have an effect on the release of adrenaline of sympathetic-vagus nerve terminal by electrical stimulation.

The above results of experiments using three different types of isolated nerve–synapse preparations lead to the conclusion that HWTX-I has presynaptic activity which effects the release of neurotransmitter from the nerve endings of both cholinergic and adrenergic synapses. This result is different from the result of Zhou et al. (1997), which suggested that huwentoxin-I blocks postsynaptic ACh receptors based on the result that D-tubocurarine and huwentoxin-I showed competitive effects in the blockade of neuromuscular transmission. We analyzed the experimental procedure of Zhou et al. In their method, there is at least one question which may lead to an uncertain result. They used the recovery time instead of the blockade time as the measurement of competition. This is an indirect measurement of competition and is not certain enough for the competition test, because the recovery time of the twitch responses of the diaphragm preparation elicited by nerve stimulation might be effected by some other factors. Even the presynaptic activity of the toxin can effect the recovery time of the twitch responses of the diaphragm preparation. The conclusion that HWTX-I is not an Ach receptor inhibitor has also been confirmed by other two results in our laboratory: (1) HWTX-I has no effect on the micro end plate potential (mEPP) of the mouse phrenic nerve–diaphragm synapse and (2) the radioisotope labeled HWTX-I can not bind to the ACh receptor of Torpedo electric organ.

The results presented here can not tell whether the exact site of action of HWTX-I is at the nerve terminal (where it might block  $\text{Ca}^{+2}$  entry or vesicle docking) or the nerve trunk (where it might block voltage  $\text{Na}^{+1}$  channels or elsewhere to block axonal conduction.). Recently, during the reviewing of this paper, we did the investigation of the effect of HWTX-I on  $\text{Ca}^{2+}$  currents in differentiated NG108-15 cells by using whole-cell patch clamp method. We found that HWTX-I could potently block high-voltage-activated  $\text{Ca}^{+2}$  channel expressed in prostaglandin E1 differentiated NG108-15 cells. The results will be published soon.

## Acknowledgements

This work was supported by Project “863” of the Science Committee of China and by National Science Foundation of China.

## References

- Arai, K., Ishima, R., Morikawa, S., Miyasaka, A., Imoto, T., Aimoto, S., Akasaka, K., 1995. Three dimensional structure of gurmardin, a sweet taste-suppressing polypeptide. *J. Biomol. NMR* 5, 297–305.
- Guo, Z.G., Rong, Q., 1989. Dissociation of cimetidine effects on inotropic and chronotropic action in cardiac anaphylaxis. *Acta Pharmacol. Sin.* 10 (2), 140–143.
- Harry, J., 1964. The site of action of sympathomimetic amines on the circular muscle strip from the guinea pig isolated ileum. *J. Pharm. Pharmacol.* 16, 332–336.
- Holak, T.A., Habazettl, J., Oschkinat, H., Otlewski, J., 1991. Structures of proteins in solution derived from homonuclear three-dimensional NOE–NOE nuclear magnetic resonance spectroscopy. High-resolution structure of squash trypsin inhibitor. *J. Am. Chem. Soc.* 113, 3196–3198.
- Lew, M.J., Flinn, J.P., Pallaghy, P.K., Murphy, R., Whorlow, S.L., Norton, R.S., Angus, J.A., 1997. Structure–Function relationships of  $\omega$ -conotoxin GVIA. *J. Biol. Chem.* 272 (18), 12014–12023.
- Liang, S.P., Zhang, D.Y., Pan, X., Chen, Q., Zhou, P.A., 1993. Properties and amino acid sequence of huwentoxin-I, a neurotoxin purified from the venom of the Chinese bird spider *Selenocosmia huwena*. *Toxicon* 31, 969–978.
- Pallaghy, P.K., Duggan, B.M., Pennington, M.W., Norton, R.S., 1993. Three dimensional structure in solution of the calcium channel blocker  $\omega$ -conotoxin. *J. Mol. Biol.* 234, 405–420.
- Pallaghy, P.K., Nielsen, K.J., Craik, D.J., Norton, R.S., 1994. A common structural motif incorporating a cystine knot and a triple-stranded  $\beta$ -sheet in toxic and inhibitory polypeptides. *Protein Sci.* 3, 1833–1839.
- Qu, Y.X., Liang, S.P., Ding, J.Z., Ma, L.B., Zhang, R.J., Gu, X.C., 1995. Proton nuclear magnetic resonance studies on Huwentoxin-I from the Venom of the Spider *S. huwena*. 1. Sequence-specific H-NMR assignment. *J. Protein. Chem.* 14, 549–557.
- Qu, Y.X., Liang, S.P., Ding, J.Z., Liu, X.C., Zhang, R.J., Gu, X.C., 1997. Proton nuclear magnetic resonance studies on huwentoxin-I from the venom of the spider *S. huwena*. 2. Three-dimensional structure. *J. Prot. Chem.* 16, 565–574.
- Zhang, D.Y., Liang, S.P., 1993. Assignment of the three disulfide bridges of huwentoxin-I, a neurotoxin from the spider *Selenocosmia huwena*. *J. Protein Chem.* 12, 735–740.
- Zhou, P.A., Xie, X.J., Li, M., Yang, D.M., Xie, Z.P., Zong, X., Liang, S.P., 1997. Blockade of neuromuscular transmission by huwentoxin-I, putified from the venom of the Chinese bird spider *Selenocosmia huwena*. *Toxicon* 35, 39–45.