ELSEVIER

European Journal of Pharmacology 454 (2002) 25–30

www.elsevier.com/locate/ejphar

The binding of BmK abT, a unique neurotoxin, to mammal brain and insect Na^+ channels using biosensor

Yong-Hua Ji [a,*], Wei-Xi Wang [a], Qiong Wang [b], Yong-Ping Huang [c]

[a] *The Key Laboratory of Neuroscience, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai 200031, PR China*

[b] *Institute of Biochemistry and Cell Biology, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, PR China*

[c] *Institute of Plant Physiology and Ecology, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, PR China*

Received 28 March 2002; received in revised form 26 August 2002; accepted 29 August 2002

Abstract

The binding properties of BmK abT (a novel neurotoxic polypeptide abT from Chinese scorpion *Buthus martensi* Karsch), a unique neurotoxin from Chinese scorpion, on mammal brain and insect sodium channels were investigated using the BIAcore assay. Results showed that BmK abT could bind to rat brain synaptosomes with an association rate constant of about 2.49×10^6 M^{-1} s^{-1} and a dissociation rate constant of about 1.57×10^{-4} s^{-1}, and to Heliothis nerve cord synaptosomes with an association rate constant of about 1.21×10^7 M^{-1} s^{-1} and a dissociation rate constant of about 0.99×10^{-3} s^{-1}. The binding of BmK abT to rat brain synaptosomes could be partially inhibited by increasing the membrane potential, but not by BmK AS (a novel active polypeptide AS from *B. martensi* Karsch), BmK IT2 (a depressant insect-selective toxin IT2 from *B. martensi* Karsch), and BmK I (an α-like anti-mammal toxin I from *B. martensi* Karsch). Binding was not modulated by veratridine. In addition, the binding of BmK abT to Heliothis nerve cord synaptosomes was significantly enhanced by increasing the membrane potential and veratridine concentration and was inhibited by BmK IT2, but not by BmK AS or BmK I. The results suggest that BmK abT binds to a distinct receptor site on mammal brain Na^+ channels and associates with a related site for depressant insect-selective toxins on insect sodium channels.

Keywords: Scorpion neurotoxin; BmK abT; Na^+ channel; Receptor binding site; BIAcore assay

1. Introduction

Voltage-dependent sodium channels are integral plasma membrane proteins responsible for the generation and propagation of action potentials in most excitable tissues. Being a critical element in excitability, sodium channels serve as specific targets for many neurotoxins. At least six neurotoxin receptor sites have been identified by direct radiolabeled toxin binding to mammalian sodium channels. Insect neuronal membranes have been shown to possess similar receptor sites (Gordon et al., 1996).

Most of the sodium channel-specific scorpion toxins are polypeptides composed of 60–76 amino acid residues (Gordon et al., 1998). Based on their pharmacological and electrophysiological characteristics, they have been divided into several principal types, α- or β-type mammal toxins, excitatory or depressant insect-selective neurotoxins (Zlotkin et al., 1985, 1993; Martin-Eauclaire and Couraud, 1995; Possani et al., 1999). Among them, α-toxins are deemed to bind to site 3 of the sodium channel and β-toxins to bind to site 4 (Cestele et al., 1995, 1998).

The Chinese scorpion *Buthus martensi* Karsch (BmK) is a species widely distributed from northwestern China to Mongolia and Korea. Up to now, at least 10 long-chain sodium channel-specific modulators have been isolated and characterized from BmK venom (Ji et al., 1994a,b, 1996, 1999). Among them, the pharmacological function of BmK AS (a novel active polypeptide AS from *B. martensi* Karsch), BmK IT2 (a depressant insect-selective toxin IT2 from *B. martensi* Karsch), and BmK I (an α-like anti-mammal toxin I from *B. martensi* Karsch), including their binding properties at mammal brain and insect synaptosomes, have been investigated extensively (Kuniyasu et al.,

* Corresponding author. Tel.: +86-21-64748057; fax: +86-21-64332445.

E-mail address: yhji@server.shcnc.ac.cn (Y.-H. Ji).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02363-4

1999; Ji et al., 1997; Jia et al., 1999; Li et al., 2000a,b; Li and Ji, 2000; Tan et al., 2001a,b; Wang et al., 2000).

BmK abT (a novel neurotoxic polypeptide abT from Chinese scorpion *B. martensi* Karsch) is a novel toxin with unique molecular and functional characteristics. It can increase the peak sodium current, slow down the inactivation of sodium channels, and prolong the action potential of dorsal root ganglion neurons, which strongly indicates that BmK abT behaves in a similar way as classical α-toxins. On the other hand, BmK abT is structurally different from classical α- and β-toxins, as well as the excitatory anti-insect toxins (with 20–30% sequence identity), but has a rather high sequence homology with the β-subtype toxins such as depressant anti-insect toxins. The functional similarity to α-toxins and the structural similarity to β-toxins suggest that BmK abT might represent a link between α- and β-toxins (Ye et al., 2000).

In the present study, we investigated the binding properties of BmK abT on both rat brain synaptosomes and insect neuronal membranes using the BIAcore approach. The possible receptor sites of BmK abT on mammal brain-type and insect sodium channel are discussed.

2. Materials and methods

2.1. Materials

BmK abT, BmK AS, BmK IT2 and BmK I were purified using previously published procedures (Ji et al., 1994a,b, 1996, 1999; Ye et al., 2000). The purity of the components was confirmed by either mass spectrometry or sequencing determination. Pepstatin A and veratridine were from Sigma. Phenylmethylsulfonylfluoride and iodoacetamide were from Wako, Japan. 1,10-Phenanthroline was obtained from Dojindo, Japan. All other reagents used were of analytical grade. Adult male Wistar rats (5–8 weeks, 250–350 g) were purchased from the Shanghai Center for Experimental Animals.

2.2. Preparation of rat brain and insect nerve cord synaptosomes

Rat brain synaptosomes were prepared according to the method described by Dodd et al. (1981). Crude insect synaptosomes were prepared from the nerve cord of Heliothis according to the method described by Lima et al. (1989). Both types of synaptosomes were suspended in a solution consisting of (in mM): choline chloride, 140; $CaCl_2$, 1.8; KCl, 5.4; $MgSO_4$, 0.8; D-glucose, 10; and HEPES, 25. The pH of the solution was adjusted to 7.4 with 2-amino-2-hydro-xymethyl-propan-1,3-diol (Tris) and used for the biosensor assay at once. A combination of proteinase inhibitors consisting of phenylmethylsulfonylfluoride (50 μg/ml), pepstatin A (1 μM), iodoacetamide (1 mM), and 1,10-phenanthroline (1 mM) was present in all buffers used in the procedure. The concentration of the final synaptosome suspension was determined using a Bio-Rad protein assay with bovine serum albumin as a standard.

All experiments conformed to the ethical guidelines described in the NIH Guide for Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.3. Immobilization and analysis with BIAcore

Ligand–receptor interaction studies and competitive binding assays were performed using a Pharmacia BIAcore instrument according to the description of Jia et al. (1999). The carboxylmethyl dextran matrix of the CM5 research grade sensor chips used in the experiments was activated by injection (40 μl) of a 1:1 (v/v) mixture of 400 mM *N*-ethyl-*N*-(dimethylaminepropyl)-carbodiimide and 100 mM *N*-hydroxy-succimide (Pharmacia). The BmK abT was immobilized onto the sensor chip by injecting 30 μl of a 200 μg/ml solution in 10 mM sodium acetate (pH 6.0) at a flow rate of 6 μl/min in HEPES-buffered saline (150 mM NaCl, 3.5 mM EDTA, 0.05% BIAcore surfactant P-20, and 10 mM HEPES; pH 7.4). The unreacted *N*-hydroxysuccinimide ester groups were quenched by injection (50 μl) of 1 M ethanolamine hydrochloride (pH 8.0; Pharmacia).

BmK abT, BmK AS, BmK IT2, and BmK I were preincubated with rat brain synaptosomes at 37 °C for 30 min and with Heliothis nerve cord synaptosomes at 22 °C for 60 min, respectively, and then injected over the sensor chips to study the competitive binding properties of BmK abT with several other BmK neurotoxins. All reaction procedures were carried out in running buffer (in mM): choline chloride, 140; $CaCl_2$, 1.8; KCl, 5.4; $MgSO_4$, 0.8; HEPES, 25; and glucose, 10 at a flow rate of 5 μl/min at 20 °C.

The binding assay was performed by injecting rat brain and Heliothis nerve cord synaptosomes over immobilized BmK abT at different concentrations. The membrane potential was modified by increasing the K^+ concentration and reducing the choline concentration (Cestele et al., 1995, 1997; Cestele and Gordon, 1998). The final concentration of [choline] plus [K^+] was 145 mM. Veratridine in different concentrations was incubated with rat brain synaptosomes at 37 °C for 30 min and with Heliothis nerve cord synaptosomes at 22 °C for 60 min, and then the complex was injected over the sensor chip carrying out the binding assay.

3. Results

3.1. The kinetics of BmK abT binding to rat brain synaptosomes

The binding of immobilized BmK abT to rat synaptosomes was monitored as resonance units (RU), which was directly proportional to the mass bound to the chip. BmK abT immobilized to the sensor chip surface was 1151.4 RU.

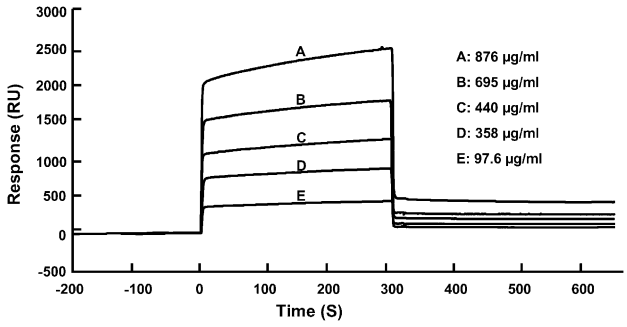

Fig. 1. BIAcore analysis of immobilized BmK abT binding to rat brain synaptosomes. Sensorgram overlays of various concentrations of synaptosomes (97.6–876 μg/ml) injected over immobilized BmK abT are shown ($n=3$). BmK abT was immobilized on a CM5 sensor chip as described in Materials and Methods. To completely dissociate bound protein, the sensor chip was regenerated with 0.1 N NaOH. These conditions allow >95% retention of the original binding activity to the immobilized BmK abT.

The kinetics of BmK abT binding were determined by injecting various concentrations of rat brain synaptosomes (97.6–876 μg/ml). The rat brain synaptosomes bound to the chip rapidly depending on the concentration gradient. Passing running buffer over the chip permitted us to monitor the first-order dissociation of synaptosomes from immobilized BmK abT. The kinetics of BmK abT binding are shown in Fig. 1. The association and dissociation rates were 2.49×10^6 M^{-1} s^{-1} and 1.57×10^{-4} s^{-1}, respectively, according to the BIA Evaluation 1.0 and 2.0 software packages analysis (Table 1).

3.2. Competitive binding of BmK abT with other BmK neurotoxins to rat brain synaptosomes

An equal quality of rat brain synaptosomes (358 μg/ml) was injected as a negative control with the binding of 240 RU. Under a certain concentration of 1.0×10^{-5} M, 92.87% binding of immobilized BmK abT was inhibited by native BmK abT, while only 27.14%, 22.45%, and 22.29% of BmK abT binding was inhibited by BmK AS, BmK I, and BmK IT2, respectively. While the concentration

Table 1
Kinetics and equilibrium of BmK abT interaction with rat brain synaptosomes by biosensor assay

Concentration of synaptosomes (μg/ml)	Association rate constant ($\times 10^6$ M^{-1} s^{-1})	Dissociation rate constant ($\times 10^{-4}$ s^{-1})	Dissociation constant ($\times 10^{-11}$ M)
876	1.85	2.60	14.1
695	1.65	1.25	7.58
440	1.97	1.17	5.95
358	2.33	1.07	4.60
97.6	4.65	1.78	3.82
Mean ± S.D.	2.49 ± 1.23	1.57 ± 0.64	7.21 ± 4.11

Data were analyzed using the BIA Evaluation 1.0 and 2.0 software packages.

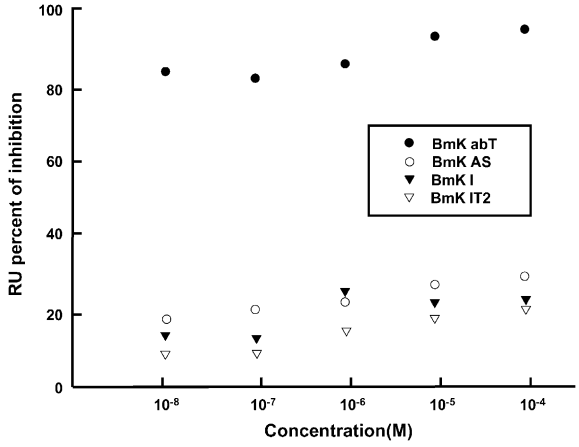

Fig. 2. The concentration–response curve of BmK abT binding to rat brain synaptosomes inhibited by different BmK neurotoxins. The freshly prepared synaptosomes were incubated with BmK abT, BmK AS, BmK I and BmK IT2 at a concentration range from 1.0×10^{-8} to 1.0×10^{-4} M at 37 °C for 30 min, and then the complex was injected over the immobilized BmK abT in binding buffer ($n=2$). The sensor chip was regenerated with 0.1 N NaOH. These conditions allow >95% retention of the original binding activity to the immobilized BmK abT. Data were analyzed as the percent binding compared with control.

of BmK neurotoxins varied from 1.0×10^{-8} to 1.0×10^{-4} M, the inhibitory effect did not vary (Fig. 2).

3.3. The kinetics of BmK abT binding to Heliothis nerve cord synaptosomes

A series of concentrations of Heliothis nerve cord synaptosomes (36.0–212 μg/ml) was injected over the immobilized sensor chip. The insect nerve cord synaptosomes bound to the chip depending on the concentration gradient. Passing running buffer over the chip permitted us to monitor the first-order dissociation of synaptosomes from immobilized BmK abT. The kinetics of BmK abT binding are shown in Fig. 3. The association and dissoci-

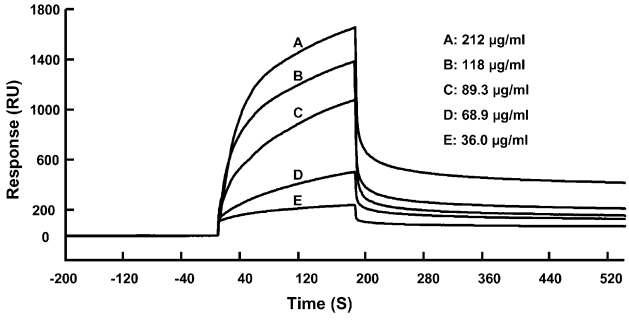

Fig. 3. BIAcore analysis of immobilized BmK abT binding to Heliothis nerve cord synaptosomes. Sensorgram overlays of various concentrations of synaptosomes (36–212 μg/ml) injected over immobilized BmK abT are shown ($n=3$). BmK abT was immobilized on a CM5 sensor chip as described in Materials and Methods. To completely dissociate bound protein, the sensor chip was regenerated with 0.1 N NaOH. These conditions allow >95% retention of the original binding activity to the immobilized BmK abT.

Table 2
Kinetics and equilibrium of BmK abT interaction with Heliothis nerve cord synaptosomes by biosensor assay

Concentration of synaptosomes (μg/ml)	Association rate constant ($\times 10^7$ M^{-1} s^{-1})	Dissociation rate constant ($\times 10^{-3}$ s^{-1})	Dissociation constant ($\times 10^{-11}$ M)
212	0.85	0.91	10.6
118	1.03	1.08	10.5
89.3	1.24	1.14	9.17
68.9	1.37	1.02	7.43
36.0	1.55	0.82	5.32
Mean ± S.D.	1.21 ± 0.28	0.99 ± 0.13	8.60 ± 2.24

Data were analyzed using the BIA Evaluation 1.0 and 2.0 software packages.

ation rates were 1.21×10^7 M^{-1} s^{-1} and 0.99×10^{-3} s^{-1}, respectively, according to the BIA Evaluation 1.0 and 2.0 software packages analysis (Table 2).

3.4. Competitive binding of BmK abT with other BmK neurotoxins to Heliothis nerve cord synaptosomes

The competitive binding assay with Heliothis nerve cord synaptosomes showed that the binding of immobilized BmK abT was completely inhibited by native BmK abT with 94.52% inhibition at 1.0×10^{-5} M. At the same applied concentration, BmK IT2 inhibited the binding dramatically by up to 74.01%; however, BmK AS and BmK I only inhibited binding by about 15.94% and 8.04%, respectively. As the concentration changed from 1.0×10^{-8} to 1.0×10^{-4} M, no obvious variation was found for the inhibitory effect of BmK AS and BmK I, whereas BmK IT2 had a biphasic inhibitory effect (Fig. 4). It showed high

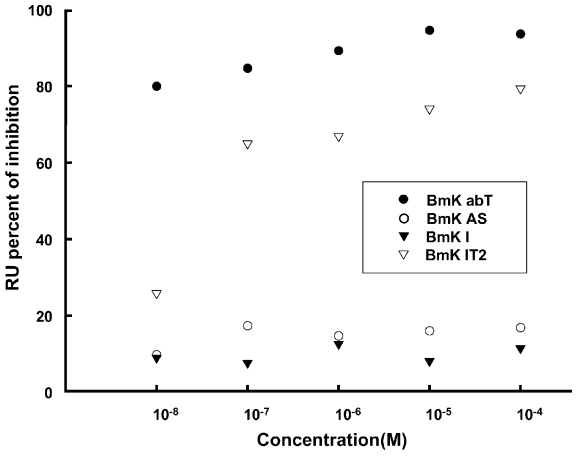

Fig. 4. The concentration–response curve of BmK abT binding to Heliothis nerve cord synaptosomes inhibited by different BmK neurotoxins. The freshly prepared synaptosomes were incubated with BmK abT, BmK AS, BmK I, and BmK IT2 over a concentration range from 1.0×10^{-8} to 1.0×10^{-4} M at 22 °C for 60 min, and then the complex was injected over the immobilized BmK abT in binding buffer ($n=2$). The sensor chip was regenerated with 0.1 N NaOH. These conditions allow >95% retention of the original binding activity to the immobilized BmK abT. Data were analyzed as the percent binding compared with control.

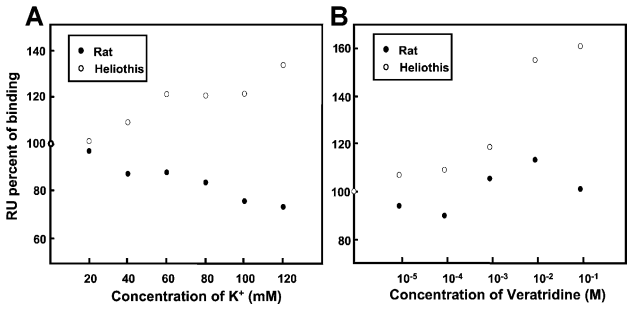

Fig. 5. Effect of membrane potential and veratridine on BmK abT binding to both synaptosomes. (A) Effect of membrane depolarization. BmK abT was incubated with rat brain synaptosomes at 37 °C for 30 min or Heliothis nerve cord synaptosomes at 22 °C for 60 min in choline-binding media containing increasing concentrations of K^+ ($n=2$). The control binding is presented as 100%. (B) Effect of veratridine. BmK abT was incubated with rat brain and Heliothis nerve cord synaptosomes containing increasing concentrations of veratridine. The control binding is given as 100% ($n=2$).

affinity with low capacity over the low concentration range from 10^{-8} to 10^{-7} M and low affinity with high capacity over the high concentration range from 10^{-6} to 10^{-4} M, which corresponds with the results of a previous study (Li et al., 2000b).

3.5. Effect of membrane depolarization or veratridine on BmK abT binding to rat brain synaptosomes and insect synaptosomes

BmK abT binding to rat brain synaptosomes could be partially inhibited when the membrane potential of synaptosomes was increased from −55 to 0 mV by increasing the concentration of K^+ from 5 to 135 mM in the binding buffer (Cestele et al., 1995, 1997; Cestele and Gordon, 1998) as shown in Fig. 5A (control binding is given as 100%). However, veratridine at concentrations ranging from 10^{-5} to 10^{-1} M seemed not to modulate the binding of BmK abT to rat brain synaptosomes (Fig. 5B). BmK abT binding to Heliothis nerve cord synaptosomes was modulated in a positive manner when the membrane potential of the synaptosomes was increased from −55 to 0 mV, as shown in Fig. 5A. Veratridine was able to promote binding, and the binding was modulated by 63.01% at the concentration of 0.1 M (Fig. 5B).

4. Discussion

This study found that BmK abT bound to rat brain synaptosomes quickly and reached an equilibrium state easily with an association rate constant of about 2.49×10^6 M^{-1} s^{-1}, five times faster than that for binding to Heliothis nerve cord synaptosomes (the association rate constant was about 1.21×10^7 M^{-1} s^{-1}). By contrast, the dissociation of BmK abT from Heliothis synaptosomes (about 0.99×10^{-3} s^{-1}) was about six times faster than that from rat synaptosomes (about 1.57×10^{-3} s^{-1}). The considerable difference in

association and dissociation rates suggests that the affinity of mammal brain sodium channels for BmK abT is higher than that of insect sodium channels. The diverse interaction modes and binding affinities may result from the structural features of binding sites of BmK abT on mammal brain and insect sodium channels.

The binding of BmK abT was modified by increasing the K^+ concentration (from −55 to 0 mV), which strongly indicates that BmK abT binding to both mammal brain and insect synaptosomes is dependent on the membrane potential (Fig. 5), as it is with classical α-toxins. However, it was unexpected that the binding of BmK abT to Heliothis nerve cord synaptosomes was enhanced, whereas binding to rat brain synaptosomes was negatively modulated by depolarization of the membrane (Fig. 5). Generally, sodium channels are present in two states, either resting or inactivated. The sodium channels were driven into an inactive state when the membrane potential was depolarized to 0 mV. Two opposing modulatory mechanisms may imply that a subtle variation between mammal brain and insect sodium channels, in which BmK abT binding receptor sites are involved directly, might lead to functional diversity of target proteins. Another possibility is that BmK abT might easily bind to Heliothis nerve cords when sodium channels are in the inactive state and to rat brain synaptosomes when sodium channels are in the resting state.

It had been demonstrated that the specific binding of BmK IT2 to rat brain synaptosomes is undetectable even in the presence of 10 μM native BmK IT2 (Li et al., 2000b). However, the specific binding of BmK AS to both rat brain and insect synaptosomes can be effectively inhibited with BmK IT2 (Li et al., 2000a). The results imply that the binding sites for BmK AS on both mammal brain and insect sodium channels might partially overlap with those of BmK IT2 although the affinity of BmK IT2 for mammal brain sodium channels was deemed to be quite weak. This study found that the binding of BmK abT to rat brain synaptosomes could not be displaced by BmK IT2 (Fig. 2), suggesting that the binding receptor site of BmK abT was different from that of BmK IT2 on mammal brain sodium channels.

The binding of BmK AS to sodium channels was shown to be similar to that of β-type toxins (Li et al., 2000a). The lack of competition of BmK AS with the binding of BmK abT to both mammal brain and insect synaptosomes (Figs. 2 and 4) indicated that BmK abT and BmK AS might bind individually to two distinct receptor sites of sodium channels. This is supported by electrophysiological data showing that BmK abT worked on sodium channels in a similar way as classical α-toxins (Ye et al., 2000).

α-Type scorpion neurotoxins have been classified into several subtypes such as classical α-toxins and α-like toxins. Classical α-toxins bind to receptor site 3 on the mammal brain sodium channel; however, the binding site of α-like toxins seems to be ambiguous (Cestele et al., 1999). The receptor site 3 has been shown to have complex allosteric interactions with neurotoxin receptor site 2, which has been shown to bind several alkaloid toxins such as veratridine (Gordon, 1997). This study found that the binding of BmK abT to rat synaptosomes was neither displaced by BmK I, an α-like neurotoxin (Ji et al., 1996) (Fig. 2), nor modulated by veratridine (Fig. 5). This indicates that the binding site of BmK abT might be different from that of classical α-toxins or α-like toxins on rat brain sodium channels.

In the case of insect sodium channels, both BmK abT and BmK AS, were found to be capable of binding, but with individual characters. Firstly, the binding affinity of BmK abT ($K_d = 8.60 \times 10^{-11}$ M) was higher than that of BmK AS ($K_d = 7.90 \times 10^{-10}$ M). Secondly, the binding of BmK abT could be modulated positively by the membrane potential, while that of BmK AS was independent of membrane potential. Thirdly, veratridine could modify BmK abT binding, but not BmK AS binding. Thus, it is possible that there are two distinct non-overlapping receptor sites for the binding of BmK abT and BmK AS on insect sodium channels.

The affinity of BmK I for insect synaptosomes was much lower ($K_d = 1.65 \times 10^{-8}$ M) than that of BmK abT. Therefore, BmK I was incompetent to inhibit the binding of BmK abT to Heliothis nerve cord synaptosomes (Fig. 4). This may be due to the lower affinity of BmK I. However, it cannot be excluded that the two toxins occupy diverse binding sites on insect sodium channels.

Finally, the binding of BmK abT to Heliothis nerve cord synaptosomes was significantly inhibited by BmK IT2 (Fig. 4). However, unlike that of BmK IT2, the binding of BmK abT to insect sodium channels could be positively modulated by depolarization and veratridine (Fig. 5). The results suggest that the receptor sites of BmK abT might be either closely related to or partially overlapping those of BmK IT2 on insect sodium channels.

On the basis of the above results, several conclusions can be drawn: (1) BmK abT is able to bind to mammal brain and insect sodium channels; (2) the receptor binding sites of BmK abT on mammal brain and insect sodium channels may be distinct in their structural and functional properties; and (3) the manner of BmK abT binding to mammal brain and insect sodium channels is different. BmK abT may bind to a distinct receptor site on mammal brain sodium channels, which is unconnected with that for BmK AS, BmK IT2 or BmK I, but which seems to be related to that for classical α-toxins. In contrast, the receptor site for BmK abT on insect sodium channels might be closely related to that for BmK IT2, a depressant insect-selective toxin.

Acknowledgements

This work was supported by National Basic Research Program of China (1999054001), partial grants from

National Nature Sciences Foundation of China (39625010) and Shanghai Metropolitan Fund for Research and Development (00JC14040). This article is also presented in memory of Professor Noboru Yanaihara.

References

Cestele, S., Gordon, D., 1998. Depolarization differentially affects allosteric modulation by neurotoxins of scorpion α-toxin binding on voltage-gated sodium channels. J. Neurochem. 70, 1217–1226.

Cestele, S., Ben, Khalifa, R.B., Pelhate, M., Rochat, H., Gordon, D., 1995. α-Scorpion toxins binding on rat brain and insect sodium channels reveal divergent allosteric modulations by brevetoxin and veratridine. J. Biol. Chem. 270, 15153–15161.

Cestele, S., Kopeyan, C., Oughideni, R., Mansuelle, P., Granier, C., Rochat, H., 1997. Biochemical and pharmacological characterization of a depressant insect toxin from the venom of the scorpion *Buthacus arenicola*. Eur. J. Biochem. 243, 93–99.

Cestele, S., Qu, Y., Rogers, J.C., Rochat, H., Scheuer, T., Catterall, W.A., 1998. Voltage sensor-trapping: enhanced activation of sodium channels by β-scorpion toxin bound to the S3-S4 loop in domain II. Neuron 21, 919–931.

Cestele, S., Stankiewicz, M., Mansuelle, P., De Waard, M., Dargent, B., Gilles, N., Pelhate, M., Rochat, H., Martin-Eauclaire, M.F., Gordon, D., 1999. Scorpion alpha-like toxins, toxic to both mammals and insects, differentially interact with receptor site 3 on voltage-gated sodium channels in mammals and insects. Eur. J. Neurosci. 11, 975–985.

Dodd, P.R., Hardy, J.A., Oakley, A.E., Edwardson, J.A., Perry, E.K., Delaunoy, J.P., 1981. A rapid method for preparing synaptosomes: comparison, with alternative procedures. Brain Res. 226, 107–118.

Gordon, D., 1997. Sodium channels as targets for neurotoxins: mode of action and interaction of neurotoxins with receptor sites on sodium channels. In: Gutman, Y., Lazarowici, P. (Eds.), Toxins and Signal Transduction. Harwood Academic Publishers, The Netherlands, pp. 119–154.

Gordon, D., Martin-Eauclaire, M.F., Cestele, S., Kopeyan, C., Carlier, E., Khalifa, R.B., Pelhate, M., Rochat, H., 1996. Scorpion toxins affecting sodium current inactivation bind to distinct homologous receptor sites on rat brain and insect sodium channels. J. Biol. Chem. 271, 8034–8045.

Gordon, D., Savarin, P., Gurevitz, M., Zinn-Justin, S., 1998. Functional anatomy of scorpion toxins affecting sodium channels. J. Toxicol., Toxin Rev. 17, 131–159.

Ji, Y.H., Mansuelle, P., Xu, K., Granier, C., Kopeyan, C., Terakawa, S., Rochat, H., 1994a. Amino acid sequence of an excitatory insect-selective toxin (BmK IT) from venom of the scorpion *Buthus martensi* Karsch. Sci. China, Ser B Chem. Life Sci. Earth Sci. 37, 42–49.

Ji, Y.H., Hattori, H., Xu, K., Terakawa, S., 1994b. Molecular characteristics of four new depressant insect neurotoxins purified from venom of *Buthus martensi* Karsch by HPLC. Sci. China, Ser B Chem. Life Sci. Earth Sci. 37, 955–963.

Ji, Y.H., Mansuelle, P., Terakawa, S., Kopeyan, C., Yanaihara, N., Hsu, K., Rochat, H., 1996. Two neurotoxins (BmK I and BmK II) from the venom of the scorpion *Buthus martensi* Karsch: purification, amino acid sequences and assessment of specific activity. Toxicon 34, 987–1001.

Ji, Y.H., Huang, H.Y., Chou, C.W., Liu, Y., Hoshino, M., Mochizuki, T., Yanaihara, N., 1997. BmK AS, an active scorpion polypeptide, enhances [^{3}H]noradrenaline release from rat hippocampal slices. Biomed. Res. 3, 257–260.

Ji, Y.H., Li, Y.J., Zhang, J.W., Song, B.L., Yamaki, T., Mochizuki, T., Hoshino, M., Yanaihara, N., 1999. Covalent structures of BmK AS and BmK AS-1, two novel bioactive polypeptides purified from Chinese scorpion *Buthus martensi* Karsch. Toxicon 37, 519–536.

Jia, L.Y., Zhang, J.W., Ji, Y.H., 1999. Biosensor binding assay of BmK AS-1, a novel sodium channel-blocking scorpion ligand on rat brain synaptosomes. NeuroReport 10, 3359–3362.

Kuniyasu, A., Kawno, S., Hirayama, Y., Ji, Y.H., Xu, K., Ohkura, M., Furukawa, K.I., Ohizumi, Y., Hiraoka, M., Nakayama, H., 1999. A new scorpion toxin (BmK PL) stimulates Ca^{2+}-release channel activity of the skeletal-muscule ryanodine receptor by an indirect mechanism. Biochem. J. 339, 343–350.

Li, Y.J., Ji, Y.H., 2000. Binding characteristics of BmK I, an alpha-like scorpion neurotoxic polypeptide, on cockroach nerve cord synaptosomes. J. Pept. Res. 56, 195–200.

Li, Y.J., Liu, Y., Ji, Y.H., 2000a. BmK AS: new scorpion neurotoxin binds to distinct receptor sites of mammal and insect voltage-gated sodium channels. J. Neurosci. Res. 61, 541–548.

Li, Y.J., Tan, Z.Y., Ji, Y.H., 2000b. The binding of BmK IT2, a depressant insect-selective scorpion toxin on mammal and insect sodium channels. Neurosci. Res. 38, 257–264.

Lima, M.E.D., Martin-Eauclaire, M.F., Hue, B., Diniz, C.R., Rochat, H., 1989. On the binding of two scorpion toxins to the central nervous system of the cockroach *Periplaneta americana*. Insect Biochem. 4, 413–422.

Martin-Eauclaire, M.F., Couraud, F., 1995. Scorpion neurotoxins: effects and mechanisms. Handbook of Neurotoxicology. Marcel Dekker, New York, pp. 683–716.

Possani, L.D., Becerril, B., Delepierre, M., Tytgat, J., 1999. Scorpion toxins specific for sodium-channels. Eur. J. Biochem. 264, 287–300.

Tan, Z.Y., Xiao, H., Mao, X., Wang, C.Y., Zhao, Z.Q., Ji, Y.H., 2001a. The inhibitory effects of BmK IT2, a scorpion neurotoxin on rat nociceptive flexion reflex and possible mechanism of modulating voltage-gated Na^+ channels. Neuropharmacology 40, 352–357.

Tan, Z.Y., Mao, X., Xiao, H., Zhao, Z.Q., Ji, Y.H., 2001b. *Buthus martensi* Karsch agonist of skeletal-muscle RyR-1, a scorpion active polypeptide: antinociceptive effect on rat peripheral nervous system and spinal cord, and inhibition of voltage-gated Na^+ currents in dorsal root ganglion neurons. Neurosci. Lett. 297, 65–68.

Wang, C.Y., Tan, Z.Y., Zhao, Z.Q., Ji, Y.H., 2000. Antihyperalgesia effect of BmKIT2, a depressant insect-selective scorpion toxin in rat by peripheral administration. Brain Res. Bull. 53, 335–338.

Ye, J.G., Wang, C.Y., Li, Y.J., Tan, Z.Y., Yan, Y.P., Li, C., Chen, J., Ji, Y.H., 2000. Purification, cDNA cloning and function assessment of BmK abT, a unique component from the Old World scorpion species. FEBS Lett. 479, 136–140.

Zlotkin, E., Kadouri, D., Gordon, D., Pelhate, M., Martin, M.-F., Rochat, H., 1985. An excitatory and a depressant insect toxin from scorpion venom both affect sodium conductance and possess a common binding site. Arch. Biochem. Biophys. 240, 677–887.

Zlotkin, E., Gurevitz, M., Fowler, E., Moyer, M., Adams, M.E., 1993. Depressant insect neurotoxin from scorpion venom: chemistry, action and gene cloning. Arch. Insect Biochem. Physiol. 22, 55–73.