The venom of scorpion *B. martensi* selectively inhibits transient outward K^+ currents in neonatal rat ventricular cells and K_v^+ channel currents in NG108-15 cells

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ABSTRACT

In this communication, the effects of the venom and/or minimolecular component of Chinese scorpion *Buthus martensi* Karsch (BmK) on nine kinds of K^+ or Cl^- channels have been scanned under voltage- and patch-clamp recording conditions. The venom showed weak blockade to voltage-gated K^+ channels in NG108-15 cells, while a reversible blocking effect of the minimolecular component on transient outward K^+ currents was distinctly observed in neonatal rat ventricular cells. Among nine channels tested, little effect was observed on the other seven channels. The present results suggest that Chinese scorpion BmK contains a novel class of active minimolecule, which selectively binds to K^+ channel family, in addition to the known polypeptides which bind to Na^+ channels.

Scorpion venoms are rich sources of various biologically active peptides. The accumulated data have demonstrated that most of scorpion neurotoxins may act as the Na^+ channel-blocking ligand. However, some neurotoxins purified from scorpion venoms of other species have been found to show K^+ channel-blocking effect or Cl^- channel-blocking effect (1, 9). All of these ligands were reported to be the minipeptides composed of 30–40 amino acids and linked by two or three disulfide bridges and have been used as valuable probes for the elucidation of structure and function of K^+ channels or Cl^- channels, as the intercellular signaling modulators (16).

Crude venom obtained by milking scorpion (BmK) electrically was purchased from an individual farm of scorpion culture in Suzhou, Jiangsu Province, China. Crude venom (0.4 g) was dissolved in 2 ml of 2 M acetic acid and centrifuged at 12,500 rpm for 25 min at 4°C. The supernatant was applied to a Sephadex G-25 (Pharmacia, U.S.A.)

column (2×100 cm) to obtain minimolecular fractions, which were collected and lyophilized.

Neuroblastoma-glioma hybrid clone NG108-15 cells were grown at 37°C under 10% CO_2 , in Dulbecco's Modified Eagle Medium (DMEM, Sigma, U.S.A.), containing 10% fetal bovine serum with HAT medium supplement (100 μ M hypoxanthine, 1 μ M aminopterin and 16 μ M thymidine). To induce neural differentiation, cells were then cultured in DMEM containing 5% fetal bovine serum with HAT medium supplement in the presence of 1 nM dibutyryl-cAMP (Yamasa, Japan) for about 10 days.

Single ventricular myocytes were isolated from neonatal rats in the following manner. Two to four-days old Wistar rats were decapitated, and the hearts were excised. After removing blood, connective tissues and atria, ventricles were minced into small pieces. They were placed into 4 ml of 0.05% trypsin-EDTA (1:250) solution (Gibco, U.S.A.) and incubated at 37°C under 5% CO_2 with stirring for 15 min. After discarding the supernatant, 3 ml of trypsin-EDTA solution was added and the mixture was stirred for 20 min. The super-

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Table 1 Sensitivity of Nine Kinds of K^+ or Cl^- Channels to BmK Venom and Its Mini-Molecular Fraction

	Cell preparations	Ion channels	Recording	Application	Concentration	Figures	Sensitivity
1	β -Cell isolated from rat pancreas	Ca^{2+} -activated K^+ channel	Patch-clamp (Inside-out)	Crude venom	0.5 mg/ml	Not shown	Without effect
2	Guinea pig Paneth's cell	cAMP-activated Cl^- channel	Patch-clamp (Whole-cell)	Crude venom	0.5 mg/ml	Not shown	Without effect
3	Guinea pig ventricular myocyte	cAMP-activated Cl^- channel	Patch-clamp (Whole-cell)	Crude venom	0.25 mg/ml	Not shown	Without effect
4	Human small intestinal epithelial cell line (Intestine 407)	Volume-sensitive Cl^- channel	Patch-clamp (Whole-cell)	Crude venom	0.5 mg/ml	Not shown	Without effect
5	Human large intestinal epithelial cell line (T84)	Outward-rectifying Cl^- channel	Patch-clamp (Inside-out)	Crude venom	0.25 mg/ml	Not shown	Without effect
				Crude venom	0.5 mg/ml	Not shown	Without effect
6	<i>Xenopus</i> oocytes	Inward rectifying Cl^- channel (Expressed in <i>Xenopus</i> oocytes)	Voltage-clamp	Crude venom	0.1 mg/ml	Not shown	Without effect
				Minimolecular fraction	0.5 mg/ml	Not shown	Without effect
7	Neonatal rat ventricular myocyte	Transient outward K^+ channel	Patch-clamp (Whole-cell)	Minimolecular fraction	< 0.5 mg/ml	Fig. 3	Reversible potent blockade
8	β -Cell isolated from rat pancreas	ATP-sensitive K^+ channel	Patch-clamp (Inside-out)	Minimolecular fraction	< 0.5 mg/ml	Not shown	Without effect
9	NG108-15 cell	Voltage-gated K^+ channel	Voltage-clamp	Crude venom	2.5 mg/ml	Fig. 1	Weak blockade

Fig. 1 Effects of the venom of scorpion *B. martensi* on Na⁺ and K⁺ currents in NG108-15 cell. (A) Representative current traces: a) control records in the absence of the venom; b) records 5 min after application of 0.25 μg/ml venom; c) records 10 min after application of 2.5 μg/ml venom. Step pulses were applied from -70 to 90 mV in 10 mV increments after a pre-pulse of -90 mV (40 ms duration). (B) Peak current-voltage relations of inward Na⁺ and outward K⁺ currents in the absence or presence of the venom: a) control peak K⁺ current in the absence of the venom; b) peak K⁺ currents 10 min after application of 2.5 μg/ml venom; c) control peak Na⁺ current in the absence of the venom; d) peak Na⁺ currents 5 min after application of 0.25 μg/ml venom; e) peak Na⁺ currents 10 min after application of 2.5 μg/ml venom

natant was transferred into 20 ml of M119 culture medium (Nissui, Japan) containing 20% newborn bovine serum. This procedure was repeated 4–5 times, and the supernatants were pooled. The cell suspension thus collected was centrifuged at 600 rpm for 1 min. Cells were resuspended in the same culture medium and maintained in culture dishes at 37°C under 5% CO₂. Cells were then used within 24 h.

Single adult guinea-pig ventricular myocytes were isolated in the manner reported (4). Human small intestinal epithelial cell line (Intestine 407) and human colonic epithelial cell line (T84) were cultured, as described (20, 21). Rat islet β cells

were cultured in RPMI-1640 supplemented with 10% fetal calf serum as described by Hayashi *et al.* (3). Paneth's cells, which were isolated from guinea-pig small intestine according to the method of McNicholas *et al.* (19), were morphologically identified in crypts. The *Xenopus* oocytes overexpressing an inwardly rectifying K⁺ channel clone, IRK1, were prepared by incubating in normal Barth's medium for 2–10 days after injecting 50 to 150 nl of the mRNA solution (0.5 ng/nl).

In oocytes, whole-cell currents were recorded by the two-microelectrode voltage clamp technique using the control bathing solution containing (mM): 90 KCl, 5 HEPES, 0.1 EGTA and 0.2 niflu-

mic acid.

In other cell species, patch-clamp whole-cell recordings were carried out, as described elsewhere (17), and cell-attached recordings were taken as described (3).

Transmembrane currents were recorded using a patch-clamp amplifier (list EPC-7 or Axopatch 200 A). The data were analyzed using an IBM-PC computer with pClamp software (Axon). All the experiments were performed at room temperature (23–25°C). The 4–7 cells were tested in each experiment.

The effects of BmK venom and the minimolecular fraction on nine kinds of K^+ or Cl^- channels obtained from different preparations were summarized in Table 1.

It was demonstrated that the venom or the minimolecular fraction at concentrations of 0.1 to 0.5 mg/ml was completely without effect on seven out of the nine channels tested. As shown in Table 1, these seven channels were: IRK1 expressed in *Xenopus* oocytes, Ca^{2+} -activated K^+ channel and ATP-sensitive K^+ channel of β -cells isolated from rat pancreas, cAMP-activated Cl^- channel in guinea-pig Paneth's cells, cAMP-activated Cl^- channel in guinea-pig ventricular myocytes, volume-sensitive Cl^- channel in a human small intestinal epithelial cell line (Intestine 407), and outward-rectifying Cl^- channel in human large intestinal epithelial cell line (T84). However, the venom abolished not only Na^+ current, but also suppressed partially voltage-gated K^+ channel currents in NG108-15 cells under voltage-clamp conditions for 10 min after administration of the venom at 2.5 μ g/ml (Fig. 1).

Likewise, under patch-clamp whole-cell conditions, the minimolecular fraction (< 0.5 mg/ml) showed a distinctly rapid blocking effect on transient outward K^+ currents, within 2 min after its administration to the preparation of neonatal rat ventricular myocytes (Fig. 2).

The blocking effect was reversible within 15 min after washout (Fig. 3).

Eight neurotoxins have been purified from BmK venom (6, 8, 11, 13). These toxins with 61–70 amino acid residues (12, 14) were demonstrated to be ligands to Na^+ channels (13). Recently, two new active polypeptides named as BmK AS and BmKAS-1, with 66 amino acids, which were purified from the same venom (10, 15, 18), show different properties from others in that both toxins stimulate selectively the binding of ryanodine to the ryanodine receptors in skeletal muscle (15),

Fig. 2 Filter pattern of the venom of scorpion *B. martensi* on Sephadex G-25 column. Column (2 × 100 cm) was equilibrated and eluted using 2 M acetic acid. About 400 mg of venom extract dissolved in 2 ml of eluent was loaded onto the column. Flow rate: 35 ml/h. Collection: 5.4 ml/tube. The minimolecular fractions (shaded portion) were collected and lyophilized.

markedly enhance intracellular noradrenaline release from rat hippocampal slices (7) and increase Na^+ conductance of NG108-15 cells (5).

It has thus been reported that scorpion venoms showed a striking K^+ or Cl^- channel-blocking effect in the various cell preparations. Up to now, at least eight K^+ channel-blocking ligands such as charybdotoxin, leiurotoxin, margatoxin, kaliotoxin and others (2, 9), and chlorotoxin as a small conductance chloride channel-blocking ligand (1) have been purified from the venom of scorpion species. These K^+ or Cl^- channel-blocking ligands have been found to be minipeptides consisting of 30–40 amino acid residues with two or three disulfide bridges. These minipeptide ligands have been served as useful tools for the study on different type of K^+ or Cl^- channels. In addition, the 'scaffold' of the minipeptide ligands from scorpion venoms seems to be a good model to build new molecules (22). The present results showed that both BmK venom and its minimolecular fraction blocked clearly the transient outward K^+ currents in neonatal rat ventricular myocytes and voltage-gated K^+ channel currents in NG108-15 cells, although the main neurotoxic components in BmK venom exhibited the Na^+ channel-blocking action (13). In conclusion, data suggest the presence of some new specific K^+ channel-blocking ligand(s) in the venom of Chinese scorpion BmK. Isolation and purification of active component(s) from BmK venom are now underway.

Fig. 3 Effects of the minimolecular fraction on the transient outward K⁺ currents in a neonatal rat ventricular myocyte. a) Control current traces in response to step pulses from -80 to $+100$ mV (in 20 -mV increments) in the absence of the minimolecular fraction. b) Current traces 55 s after application of about 0.45 mg/ml the minimolecular fraction. c) Current traces 5 min after application of about 0.45 mg/ml the minimolecular fraction. d) Current traces 15 min after washout of the minimolecular fraction. e) Comparison among the above traces recorded at $+100$ mV

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