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THE MYTH OF SCORPION SUICIDE: ARE SCORPIONS INSENSITIVE TO THEIR OWN VENOM?

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Summary

The resistance of the scorpion *Androctonus australis* to its own venom, as well as to the venom of other species, was investigated. A comparison of the electrical and pharmacological properties of muscle and nerve fibres from *Androctonus australis* with those from the crayfish *Procambarus clarkii* enabled us to understand the lack of effect of scorpion venom (110–180 μg ml^{-1}) and purified toxins, which are active on voltage-gated Na^+ and K^+ channels, Ca^{2+}-activated K^+ channels, on scorpion tissues. Voltage-clamp experiments showed that peptide K^+ channel blockers from scorpion and snake have no effect on currents in muscle and nerve fibres from either scorpions or crayfish. The scorpion toxin kaliotoxin (KTX), a specific blocker of Kv1.1 and Kv1.3 K^+ channels, had no effect on muscle fibres of *A. australis* (2 μmol l^{-1}) or *P. clarkii* (400 nmol l^{-1}). Similarly, charybdotoxin (ChTX) had no effect on the muscle fibres of *A. australis* (10 μmol l^{-1}) or *P. clarkii* (200 nmol l^{-1}) and neither did the snake toxin dendrotoxin (DTX) at concentrations of 100 nmol l^{-1} in *A. australis* and 200 nmol l^{-1} in *P. clarkii*. These three toxins (KTX, ChTX and DTX) did not block K^+ currents recorded from nerve fibres in *P. clarkii*. The pharmacology of the K^+ channels in these two arthropods did not conform to that previously described for K^+ channels in other species. Current-clamp experiments clearly indicated that the venom of *A. australis* (50 μg ml^{-1}) had no effect on the shape of the action potential recorded from nerve cord axons from *A. australis*. At a concentration of 50 μg ml^{-1}, *A. australis* venom greatly prolonged the action potential in the crayfish giant axon. The absence of any effect of the anti-mammal α-toxin AaH II (100 nmol l^{-1}) and the anti-insect toxin AaH IT1 (100 nmol l^{-1}) on scorpion nerve fibres revealed strong pharmacological differences between the voltage-gated Na^+ channels of scorpion and crayfish. We conclude that the venom from *A. australis* is pharmacologically inactive on K^+ channels and on voltage-sensitive Na^+ channels from this scorpion.

Key words: toxin, peptide, K^+ current, Na^+ current, Ca^{2+} current, crayfish, scorpion, muscle fibre, nerve fibre, *Androctonus australi*, *Procambarus clarkii*.

Introduction

Scorpions appeared in the middle Silurian Period and may thus be seen as living fossils within the arthropods (Jeram, 1990). It is well-documented that scorpions can survive a variety of adverse experimental protocols (e.g. irradiation) and very harsh environmental conditions (famine and drought) (Lot, 1973). According to legend, scorpions commit suicide by stinging themselves. However, contrary to this popular opinion, it has been reported that scorpions are resistant to the powerful venom that they use to immobilise their prey (Shulov and Levy, 1978; Zlotkin *et al.* 1972). Several toxic molecules in scorpion venom target ion channels and disturb the electrophysiological properties of excitable cells. These molecules belong to various families (for a review, see Martin-Eauclaire and Couraud, 1995). The first family to be described comprises miniproteins which act by binding to voltage-sensitive Na^+ channels and affecting their gating properties (for a review, see Catterall, 1995). A second family contains shorter polypeptides characterised as potent blockers of K^+ channels (for a review, see Garcia *et al.* 1991; Miller, 1995). These block two major classes of K^+ channel, voltage-gated (Kv, and particularly the Kv1 family) and Ca^{2+}-activated K^+ channels of various conductances (small, SK; intermediate, IK; large, BK_{Ca}) with affinities that are sometimes in the picomolar range. In addition, several new pharmacological properties of scorpion venoms have recently been described as a result of the interaction of peptides with ryanodine receptors or Cl^- channels (DeBin *et al.* 1993; Valdivia *et al.* 1992; Zamudio *et al.* 1997).

The resistance of scorpions to their own venom has yet to be determined experimentally. When venom or purified toxins (AaH IT1 and AaH II) from the scorpion *Androctonus australis* (*A. australis*) were injected into this species of scorpion, no toxic effects were observed (M. F. Martin-Eauclaire, personal observation), suggesting that the animals may be resistant to their own venom. Our aim was to investigate the mechanisms involved in this resistance. The fact that ion channels are the main targets of scorpion toxins suggests that in scorpions these channels may be insensitive to neurotoxins. This possibility was investigated by recording the effects of scorpion venoms and purified scorpion toxins on ion currents in the muscle tissue and nerve cord of the North African scorpion *A. australis* and comparing these effects with those in another arthropod, the crayfish *Procambarus clarkii*.

Materials and methods

Experiments were carried out with the scorpion (*Androctonus australis* Hector) and the crayfish (*Procambarus clarkii* Girard). Scorpions were collected from the area around Tozeur, Tunisia, and supplied by Professor H. Rochat (Université de la Méditerranée, France). Crayfish were obtained from a commercial supplier (Château Garreau, 'La Bastide d'Armagnac'). Scorpion venom was obtained by electrical stimulation of two scorpion species: *A. australis* from Tunisia and *Tityus serrulatus* from Brazil. Charybdotoxin (ChTX) and iberiotoxin (IbTX) were purchased from Bachem AG. Kaliotoxin (KTX) was synthesised in the laboratory as described by Romi *et al.* (1993). Dendrotoxin (DTX) was purified from the venom of the snake *Dendroaspis angusticeps* (Harvey and Karlson, 1980) and the scorpion toxins (AaH II and AaH IT1) from the venom of *A. australis* (Martin-Eauclaire and Rochat, 1986). The activity of the venom and toxins used in this study was verified routinely in the laboratory.

Crayfish axon voltage-clamp experiments

The *in vitro* preparations were kept at 9 °C using a Peltier cooler (Sqalli-Houssaini *et al.* 1991). Voltage-clamp experiments were carried out on *in vitro* preparations of crayfish axons as described by Cattaert and Lebrun (1993). The ventral nerve cord, from the fourth thoracic ganglion to the first abdominal ganglion, was isolated and pinned dorsal side up to a Sylgard-lined Petri dish (diameter 4 cm). The connectives between the fifth thoracic ganglion and the first abdominal ganglion were desheathed. The range of the clamp was restricted to a short segment of axon by mechanically isolating a 1–3 mm portion of the axon by squeezing the desheathed nerve cord with wire staples pinned to the Sylgard. Double-electrode voltage-clamp experiments were then performed on this restricted length of axon.

Crayfish muscle voltage-clamp experiments

Voltage-clamp experiments were carried out on *in vitro* muscle fibre preparations from crayfish using the procedure described by Araque *et al.* (1995). Opener muscles from the propodite of the first walking leg of the crayfish were isolated and transferred to a superfusion chamber (2 ml). Small crayfish (less than 5 cm long) with short muscle fibres (less than 400 μm long) were used because they gave better space-clamp characteristics.

Scorpion axon current-clamp experiments

Approximately 6 cm of the ventral nerve cord was dissected from the scorpion. Lengths of nerve cord 2 cm long were pinned dorsal side up to a Sylgard-lined Petri dish (4 cm diameter). Pronase (from Sigma) was used to partially digest the connective tissue surrounding the axons. It was only possible to perform single-electrode current-clamp experiments because the axons in the ventral nerve cord of scorpions have small diameters (Terakawa *et al.* 1989).

Scorpion muscle voltage-clamp experiments

The procedure used to dissect muscle fibres from the pedipalp claw was adapted from that of Gilai and Parnas (1970). The propodite was cut, and the closer muscles and their proximal tendon were exposed and dissected out. The tendon was then pinned onto a Sylgard-lined Petri dish (4 cm diameter), together with the propodite cuticle.

Electrodes and recordings

For voltage-clamp experiments, glass micropipettes with a resistance of 2–6 MΩ were filled with 3 mol l^{-1} KCl. Current-clamp recordings from scorpion axons were obtained using 30–40 MΩ glass micropipettes filled with 3 mol l^{-1} KCl. A voltage-clamp amplifier (Axoclamp 2A, Axon instruments) was used either in the bridge mode or in the two-electrode voltage-clamp mode, with probes of ×0.1 (for voltage) or ×1 or ×10 (for current). Command pulses were controlled by a programmable eight-channel stimulator from A.M.P.I.

Data were stored on a digital tape recorder (Biologic DTR 1802) and displayed on a four-channel digital oscilloscope (Yokogawa). Data acquisition and analysis were controlled by a PC computer connected to an analog/digital interface (CED 1401, Cambridge Electronic Design), using CED's SIGAV program for superimposing and averaging the voltage and current traces triggered.

Physiological solutions

The physiological solution for the scorpion nerve cord preparation was as described by Padmanabhanaidu (1967) (in mmol l^{-1}): 147 NaCl, 1.7 KCl, 6.1 $CaCl_2$, 10.4 $MgCl_2$ and 3 Tris, pH 7.3. The physiological solution for the scorpion muscle preparation was as described by Gilly and Scheuer (1993) (in mmol l^{-1}): 250 NaCl, 7.7 KCl, 10 $MgCl_2$ and 10 Tris, pH 7.1.

The bathing solution for the crayfish nerve cord preparation contained (in mmol l^{-1}): 195 NaCl, 5.5 KCl, 13.5 $CaCl_2$, 2.5 $MgCl_2$ and 10 Tris, pH 7.6; that for the crayfish muscle fibres contained (in mmol l^{-1}): 210 NaCl, 5.4 KCl, 16.1 $MgCl_2$ and 10 Tris, pH 7.2.

Pharmacology

In some experiment, tetraethylammonium chloride hydrate (TEA-Cl; at concentrations of 150 mmol l^{-1} for muscle fibre experiments and 100 mmol l^{-1} for nerve fibre experiments) or 4-aminopyridine (4-AP; 0.5 mmol l^{-1}) was added to the saline in equimolar exchange with NaCl. In some experiments, the Na^+ channel blocker tetrodotoxin (TTX) (10^{-5} mol l^{-1}) was added to the saline. TTX, TEA^+ and 4-AP were all purchased from Sigma.

Results

The effects of scorpion venom and toxins on scorpion muscle fibres

In normal saline, Ca^{2+} spikes were routinely observed in single-electrode current-clamp recordings (Fig. 1A). The characteristics of the action potential in scorpion muscle fibres have been described previously (Gilly and Scheuer, 1993). Since twitches produced by these Ca^{2+} spikes frequently caused damage to the muscle fibre, a Ca^{2+}-free solution was used to reduce the amplitude of the Ca^{2+} spike, although some contractions still occurred with large depolarising currents. Scorpion muscle fibres are very fragile, and contractions with small amplitudes may affect voltage-clamp recordings. It was, therefore, very difficult to use the same muscle fibre for control and drug application experiments. When the control and the test were performed on different fibres, several muscle fibres were tested under each set of conditions to ensure the accuracy of the results (Fig. 1). Data were reproducible, although there was some variation in kinetics and amplitude. In voltage-clamp experiments in normal saline, inward currents were due solely to Ca^{2+} influx (Gilly and Scheuer, 1993). In all the muscle fibres tested (*N*=23), scorpion venoms and toxins failed to block outward K^+ currents, even at concentrations that totally inhibit these currents in mammals (Grissmer *et al.* 1994) and molluscs (Crest *et al.* 1992). An inward rectifier K^+ current was observed in crayfish muscle fibres (see Figs 3, 4), but not in *A. australis* muscle fibres (Figs 1, 2). At a concentration of

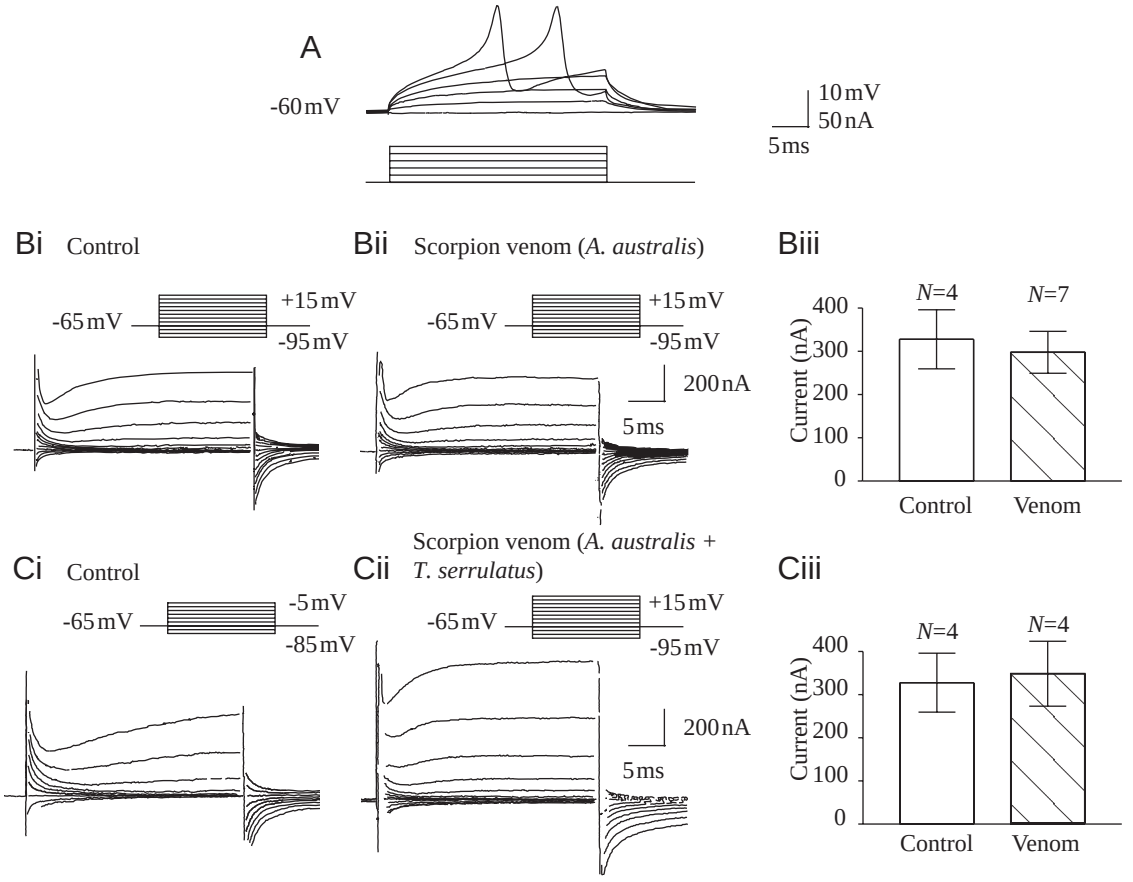

Fig. 1. Absence of effect of scorpion venom from *Androctonus australis* and *Tityus serrulatus* on scorpion muscle fibre K^+ channels. (A) Current-clamp recordings from a closer muscle fibre using graded depolarising current pulses (steps of 10 nA). (B) Voltage-clamp recordings in control solution (i) and in the presence of scorpion venom (110 μg ml^{-1}) from *A. australis* (ii). Data are from a single muscle fibre for each experiment. (C) Voltage-clamp recordings in control solution (i) and in the presence of a mixture of 110 μg ml^{-1} *A. australis* scorpion venom and 30 μg ml^{-1} *T. serrulatus* scorpion venom (ii). Data are from different muscle fibres. The membrane potential was held at −65 mV and stepped for 30 ms to potentials from −95 mV to +15 mV in increments of 10 mV to produce each set of current recordings. The steady-state currents obtained in several experiments with a voltage step from −65 mV to −5 mV are reported in the histograms (Biii and Ciii). Vertical bars indicate the standard error of the mean (S.E.M.).

110 μg ml^{-1}, *A. australis* venom did not block outward currents in scorpion muscle fibres (Fig. 1B). This concentration is usually sufficient for effective blocking; 45 μg ml^{-1} *A. australis* venom totally blocks the IK_{Ca} currents recorded in mollusc neurones (Crest *et al.* 1992). The addition of *T. serrulatus* venom (30 μg ml^{-1}) also failed to block the outward currents (Fig. 1C). However, both Ba^{2+} (10 mmol l^{-1}) (Table 1) and TEA^+ (150 mmol l^{-1}) (see Fig. 2D) blocked the outward currents, indicating that these currents were carried by K^+. The kinetics of this outward current indicates that it probably involves delayed rectifiers (Kv channels), especially in the steady state. As there was no Ca^{2+} in the solution, Ca^{2+} spikes and Ca^{2+}-dependent K^+ currents were undetectable in most of our recordings.

There may be two reasons for the lack of effect of the scorpion venoms: either the concentration of K^+ blockers was too low or scorpion K^+ channels are truly insensitive. Scorpion venoms (*A. australis* and *T. serrulatus*) contain very low concentrations (0.1 % of total proteins) of specific K^+ channel blockers such as kaliotoxin (KTX) (Blaustein *et al.* 1991). We therefore tested the effects of high concentrations of KTX and other specific K^+-channel-blocking peptides (ChTX and DTX). The results (Fig. 2) were obtained using the same fibres for both control and toxin application experiments. None of the

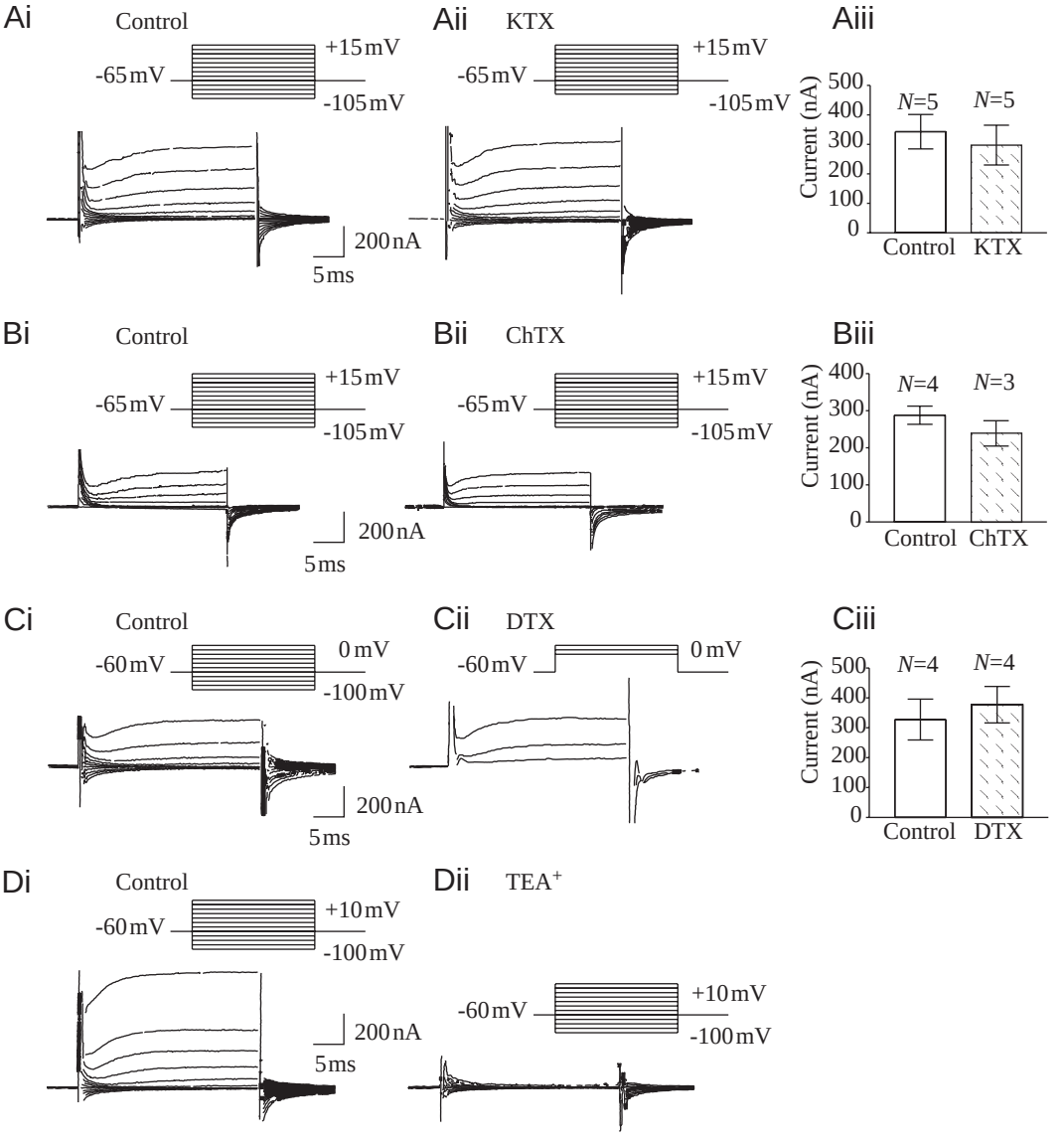

Fig. 2. Absence of effect of K^+ channel toxin blockers on scorpion muscle fibres. The scorpion toxins kaliotoxin (KTX) and charybdotoxin (ChTX) had no effect on total current. Currents in control solution and with 100 nmol l^{-1} KTX (A) and 10 μmol l^{-1} ChTX (B). The snake toxin dendrotoxin (DTX; 100 nmol l^{-1}) did not block K^+ currents (C). In each experiment, the control test and drug assay were performed on the same muscle fibre. The steady-state currents obtained in several experiments with a voltage step from –65 mV to –5 mV (A,B) and from –60 mV to 0 mV (C) are reported in the histograms (Aiii, Biii and Ciii). Vertical bars indicate the S.E.M. (D) Outward K^+ currents are blocked by 150 mmol l^{-1} TEA^+. For each experiment, the holding potential and the range of voltage pulses are reported in the corresponding insets.

Table 1. *Comparison of the effects of scorpion venoms and K^+ channel blockers on the muscle fibres of scorpion and crayfish*

		Venom of *Androctonus australis*	Venom of *Tityus serrulatus*	TEA^+	4-AP	Ba^{2+}	KTX	ChTX	IbTX	DTX
Muscle fibre of scorpion	Concentrations tested	110 μg ml^{-1}	30 μg ml^{-1}	150 mmol l^{-1}	ND	10 mmol l^{-1}	100 nmol l^{-1} 200 nmol l^{-1} 2 μmol l^{-1}	10 μmol ml^{-1}	ND	100 nmol l^{-1}
	Effect	None	None	100 % of K^+ current blocked		100 % of Kv channels blocked	None	None		None
Muscle fibre of crayfish	Concentrations tested	140 μg ml^{-1} 180 μg ml^{-1}	ND	150 mmol l^{-1}	0.5 mmol l^{-1}	ND	200 nmol l^{-1} 400 nmol l^{-1}	200 nmol l^{-1}	40 nmol l^{-1}	200 nmol l^{-1}
	Effect	None		80 % of K^+ current blocked	100 % of Kv channels blocked		None	None	None	None

ND, not determined; TEA^+, tetraethylammonium; 4-AP, 4-aminopyridine; KTX, kaliotoxin; ChTX, charybdotoxin; IbTX, iberiotoxin; DTX, dendrotoxin.

Table 2. *Comparison of the effects of scorpion venoms and K^+ and Na^+ channel blockers on the nerve fibres of scorpion and crayfish*

		Venom of *Androctonus australis*	TEA^+	TTX	KTX	ChTX	DTX	AaH II	AaH IT1
Nerve fibre of scorpion	Concentrations tested	50 μg ml^{-1}	100 mmol l^{-1}	10 μmol l^{-1}	50 nmol l^{-1} 100 nmol l^{-1} 2 μmol l^{-1}	50 nmol l^{-1}	ND	100 nmol l^{-1}	100 nmol l^{-1}
	Effect	None	100 % of K^+ currents blocked	100 % of K^+ currents blocked	None	None		None	None
Nerve fibre of crayfish	Concentrations tested	50 μg ml^{-1}	100 mmol l^{-1}	ND	100 nmol l^{-1} 200 nmol l^{-1}	200 nmol l^{-1}	200 nmol l^{-1}	ND	ND
	Effect	Na^+ currents broadened, no effect on K^+ currents	100 % of K^+ currents blocked		None	None	None		

ND, not determined; TEA^+, tetraethylammonium; TTX, tetrodotoxin; KTX, kaliotoxin; ChTX, charybdotoxin; DTX, dendrotoxin; AaH II, antimammalian alpha-toxin AaH II; AaH IT1, anti-insect toxin AaH IT1.

blockers affected the outward currents, even at concentrations that usually block Kv channels. Kaliotoxin, which blocks vertebrate Kv1.1 and Kv1.3 channels in the picomolar range (Grissmer *et al.* 1994; Aiyar *et al.* 1995), had no effect at a concentration of either 100 nmol l^{-1} (Fig. 2A) or 2 μmol l^{-1} (Table 1). Charybdotoxin, which blocks vertebrate Kv and BK_{Ca} channels in the nanomolar range (Smith *et al.* 1986; Knaus *et al.* 1994), had no effect on the current at concentrations up to 10 μmol l^{-1} (Fig. 2B). Dendrotoxin, which completely blocks vertebrate Kv1.1 and Kv1.2 channels in the nanomolar range (Rehm, 1991), is totally inactive against the outward currents of scorpion muscle, even at a concentration of 100 nmol l^{-1} (Fig. 2C).

The effects of scorpion venom and toxins on crayfish muscle fibres

The effects of scorpion toxins on the muscle fibres of crayfish were examined to test whether the lack of effect on scorpion muscle fibres was peculiar to *A. australis* or common to other arthropods. Crayfish muscle fibres have more currents than scorpion muscle fibres, including a fast inward current identified as a Ca^{2+} current by Mounier and Vassort (1975), a fast transient outward current shown to be a BK_{Ca} current (Araque and Buño, 1995) and an inwardly rectifying current activated by hyperpolarised potentials (Araque *et al.* 1995). Crude venom from *A. australis* had no apparent effect on any of these currents at either of the concentrations tested (140 and 180 μg ml^{-1}; Table 1). The results obtained with 140 μg ml^{-1} *A. australis* venom are shown in Fig. 3A. However, TEA^+ (150 mmol l^{-1}) and 4-AP (0.5 mmol l^{-1}) blocked most of the outward currents (Fig. 3B,C).

K^+ channel blockers derived from scorpion toxins also had no effect on crayfish muscle fibres. Kaliotoxin was totally inactive on the steady-state outward current (Kv) even at a concentration of 400 nmol l^{-1} (Fig. 4A), and charybdotoxin had no effect on either current, even at a concentration of 200 nmol l^{-1} (Fig. 4B). In the recordings shown in Fig. 4, Ca^{2+} and BK_{Ca} currents are transient: the Ca^{2+} current is a fast inward current followed by a small transient rapid outward current (BK_{Ca}); the characteristic outward delayed rectifier develops more slowly. The highly specific BK_{Ca} blocker IbTX (K_d=1.2–1.7 nmol l^{-1}; Candia *et al.* 1992; Giangiacomo *et al.* 1993) was also inactive at concentrations up to 40 nmol l^{-1} (see inset in Fig. 4C), although the inward Ca^{2+} current was smaller in some experiments. In crayfish muscle fibres, as in those of scorpion, the snake toxin DTX had no effect on the voltage-gated K^+ currents, even at a high concentration (200 nmol l^{-1}, Fig. 4D).

The effects of venoms and toxins on scorpion and crayfish muscle fibres are summarised in Table 1. All the substances tested normally block vertebrate K^+ channels; however, none of the well-characterised toxins except classic ligands such as TEA^+ and 4-AP, which are less specific, had any detectable effect on K^+ currents recorded under voltage-clamp conditions in crayfish and scorpion. These results were highly reproducible (see Table 1 and histograms in Figs 1, 2 and 4).

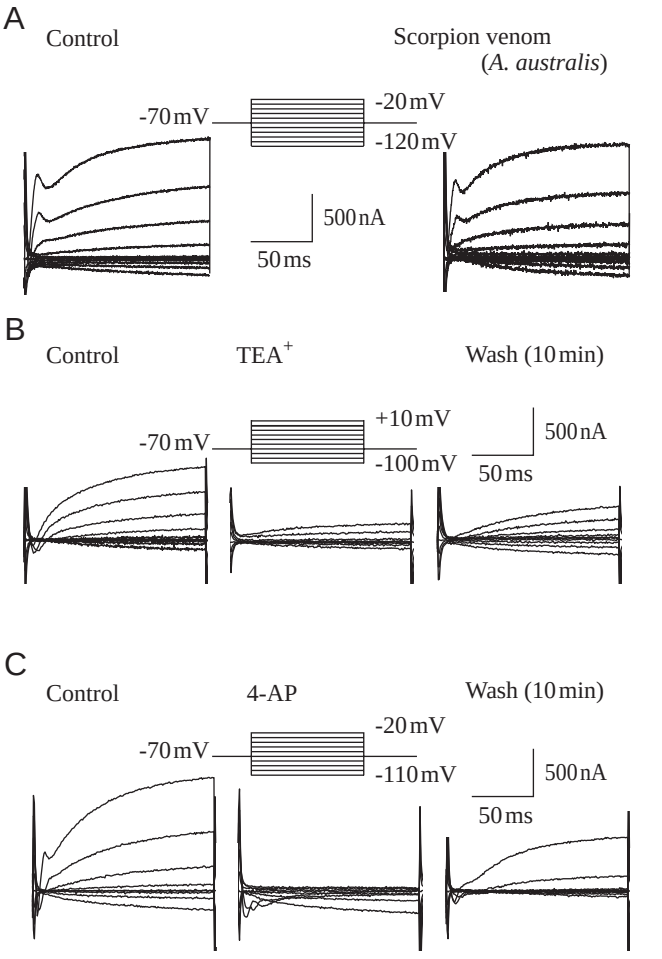

Fig. 3. Effect of scorpion venom, tetraethylammonium (TEA^+) and 4-aminopyridine (4-AP) on crayfish muscle fibres. (A) Voltage-clamp recordings from an opener muscle fibre in control conditions and in the presence of 140 μg ml^{-1} *Androctonus australis* scorpion venom. (B) Blockade of Kv channels by 150 mmol l^{-1} TEA^+. (C) Blockade of Kv channels by 0.5 mmol l^{-1} 4-AP (C). The holding potentials and the range of voltage pulses are given in the insets.

The effects of scorpion venom and toxins on scorpion nerve fibres

The ventral nerve cord of the scorpion has no giant fibres like those of some crustaceans and insects. Moreover, anatomical studies of cross sections of the nerve cord of *A. australis* showed that the axons were all of small diameter (less than 7 μm, data not shown). These structural characteristics of scorpion nerve fibres made insertion of the microelectrodes a very delicate operation and, therefore, very few two-electrode voltage-clamp experiments were performed; since such recordings were rarely stable, only current-clamp recordings are reported here. However, we tested the effect on nerve conduction by simultaneously taking both intracellular and extracellular *en passant* recordings. *A. australis* crude venom (50 μg ml^{-1}) failed to affect action potentials (Fig. 5A), whereas the spikes were totally and rapidly (in under 2 min) suppressed in the presence of 10 μmol l^{-1} TTX (Fig. 5B).

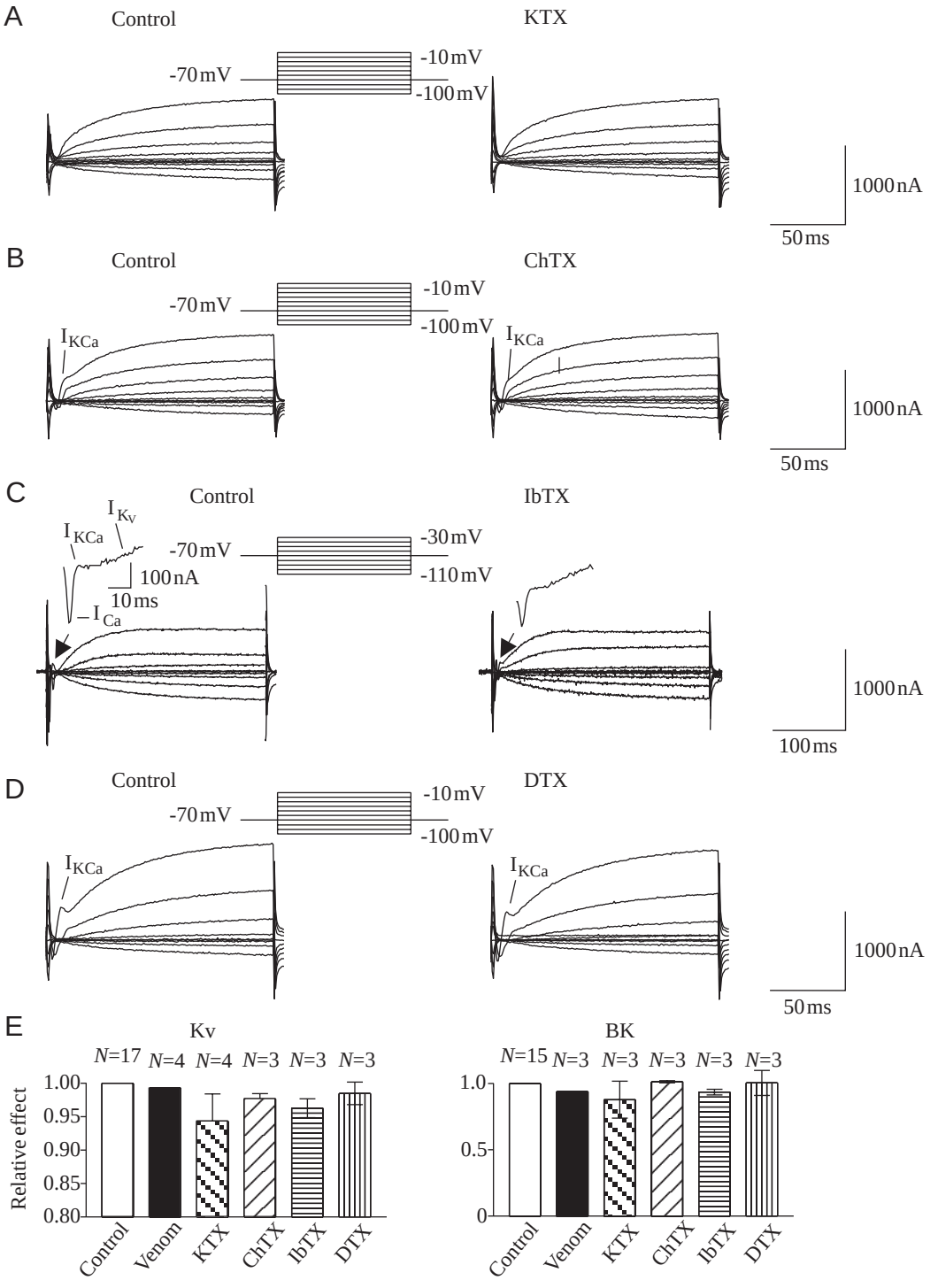

Fig. 4. Absence of any effect of K^+ channel blockers on crayfish muscle fibres. (A–C) The scorpion toxins kaliotoxin (KTX, 400 nmol l^{-1}), charybdotoxin (ChTX, 200 nmol l^{-1}) and iberiotoxin (IbTX, 40 nmol l^{-1}) had no effect on total currents. (D) The snake toxin dendrotoxin (DTX; 200 nmol l^{-1}) did not block the K^+ currents recorded. In each experiment, the control test and drug assay were performed on the same muscle fibre. The holding potentials and the range of voltage pulses are reported in the corresponding insets. (E) The steady-state currents obtained with a voltage step from −70 mV to −10 mV (A,B,D) or to −30 mV (C) are reported in the histograms. Venom, from *A. austrialis* (140–180 μg ml^{-1}). Vertical bars indicate the S.E.M. Kv, voltage-dependent K^+ channel; BK, large-conductance Ca^{2+}-activated K^+ channel.

The anti-mammalian scorpion toxin AaH II, the most potent scorpion α-toxin affecting the inactivation process of voltage-dependent mammalian Na^+ channels, had no effect on scorpion nerve fibres even at a concentration of 100 nmol l^{-1} (Fig. 5C). Neither the shape of the spike nor the conduction time was modified by AaH II. Similar results were obtained with the

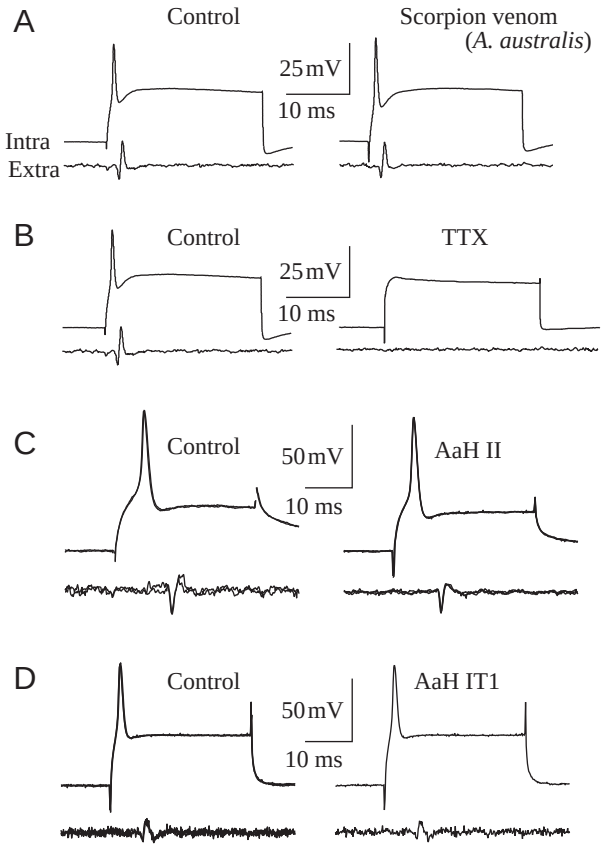

Fig. 5. Absence of effect of scorpion venom and toxins able to modulate voltage-sensitive Na^+ channels on scorpion nerve fibres. Current pulses (+10 nA) were injected into fibres from the ventral nerve cord (Intra, current-clamp recording) and the corresponding action potential was recorded *en passant* on the nerve cord (Extra). (A) Scorpion venom (*Androctonus australis*, 50 μg ml^{-1}) did not change the properties or shape of the spike. (B) Spikes disappeared in the presence of 10 μmol l^{-1} of tetrodotoxin (TTX, a voltage-sensitive Na^+ channel blocker). (C,D) The properties and shape of the spike were unaffected by the scorpion toxins AaH II (C, 100 nmol l^{-1}) and AaH IT1 (D, 100 nmol l^{-1}).

scorpion anti-insect toxin AaH IT1 (100 nmol l^{-1}, Fig. 5D), which causes a slow progressive depolarisation of the membrane potential and repetitive firing of action potentials in insects axons (Pelhate and Zlotkin, 1982). We also tested KTX (at concentrations up to 2 μmol l^{-1}) and ChTX (50 nmol l^{-1}). These two K^+ channel blockers had no effect on scorpion nerve fibres (Table 2). In contrast, in the presence of TEA^+ (100 mmol l^{-1}), spikes were larger as a result of the K^+ current being blocked (Table 2). This change in the shape of the action potential shows that the K^+ channels involved are accessible to drugs.

The effects of scorpion venom and toxins on crayfish nerve fibres

Scorpion venom (*A. australis*) slowed the inactivation of the Na^+ channel of crayfish nerve fibre (Fig. 6A), as has previously been demonstrated for the Na^+ channel of frog nerve fibres (Adam *et al.* 1966). This is due exclusively to the α-toxins in this venom, which are very effective against Na^+ channels (Benoit and Dubois, 1987; for a review, see Martin-Eauclaire and Couraud, 1995). There was no effect on the steady-state outward K^+ current of the crayfish (Fig. 6A).

The toxins active against K^+ channels were tested on the nerve fibre: KTX (up to 200 nmol l^{-1}), ChTX (up to 200 nmol l^{-1}) and DTX (up to 200 nmol l^{-1}) were found not to block Kv (Fig. 6B–D; Table 2) and had no effect on the K^+ currents. In the presence of TEA^+ (100 mmol l^{-1}), however, outward K^+ currents were almost totally blocked (Fig. 6E).

Discussion

This study demonstrates that scorpion venoms from *A. australis* and *T. serrulatus* and some of the major toxins purified from them (AaH II, AaH IT1, KTX, ChTX and IbTX, which act specifically on various types of ion channel) have almost no effect on scorpion ion channels, even at concentrations that dramatically alter the K^+ or Na^+ conductances of nerve or muscle fibres from other animals. The concentrations of toxins used were 100–1000 times higher than the affinity reported for their receptors in vertebrate and invertebrate tissues. Thus, the lack of any effect shows that scorpion ion channels do not conform to the pharmacology previously described for the Na^+ and K^+ channels in muscle or nervous system preparations from frogs, crustaceans, insects, rats and mice (Hille, 1992; Latorre *et al.* 1989). This conclusion is supported by preliminary experiments showing that injections, under the second post-abdominal ring, of crude adult *A. australis* venom (125 μg, 25×LD_{50} for mice), AaH II (30 μg, 120×LD_{50} by subcutaneous injection or 60 000×LD_{50} by intracerebroventricular injection in mice) or AaH IT1 (20 μg, 50 000×LD_{50} for the fly *Musca domestica* or 2×LD_{50} to 4×LD_{50} for the aquatic crustacean *Acanthonyx lunulatus*; De Dianous *et al.* 1987) do not cause paralysis or other signs of toxicity. These preliminary results suggest that the scorpion is very resistant to its own venom.

Drug accessibility in the preparations

The connective tissue around the nerve fibres in the crayfish and especially in the scorpion may limit the accessibility of the target to large molecules. Nevertheless, scorpion toxins such as AaH I, AaH II and Ts VII (the main beta-toxin from *Tityus serrulatus* scorpion venom), which are active against mammalian Na^+ channels (Martin-Eauclaire and Rochat, 1986; De Lima *et al.* 1986), and various toxins such as AaH IT1 (an anti-insect toxin from the scorpion *Androctonus australis*), Lqq IT2 (an anti-insect toxin from the scorpion *Leiurus quinquestriatus quinquestriatus*) or Lqh αIT (the *Leiurus quinquestriatus hebraeus* alpha anti-insect toxin), which are specific for insect Na^+ channels, have a strong effect on the Na^+ channels of crayfish (Rathmayer *et al.* 1977; Romey *et al.* 1976) and cockroach (De Lima *et al.* 1989; Babu *et al.* 1971; D'Ajello *et al.* 1972; Pelhate and Zlotkin, 1982). Light microscopy showed a thin layer of connective tissue surrounding the axons and bundles (data not shown). Enzymes

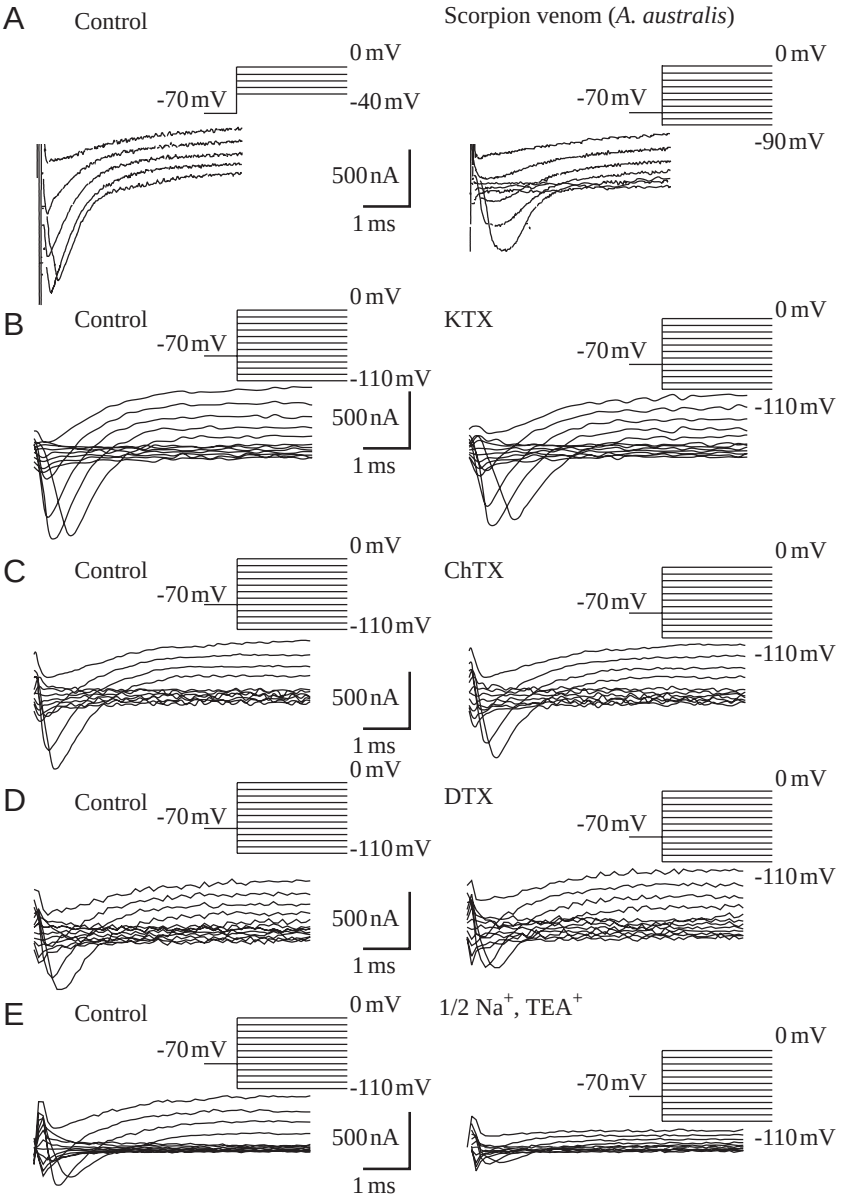

Fig. 6. Effects of scorpion venom and peptide toxins on crayfish nerve fibres. Total current recordings for several depolarising potentials are shown. (A) Scorpion venom (*Androctonus australis*, 50 μg ml^{-1}) increased the width of the inward current peak (Na^+ currents). (B–D) Kaliotoxin (KTX, 200 nmol l^{-1}), charybdotoxin (ChTX, 100 nmol l^{-1}) and dendrotoxin (DTX, 100 nmol l^{-1}) had no effect on total current. (E) Blockage of Kv channels by 100 mmol l^{-1} tetraethylammonium (TEA^+). The inward (Na^+) current was reduced by equimolar exchange of TEA^+ with NaCl. The membrane potential was held at −70 mV and stepped for 10 ms to potentials from −40 mV to 0 mV (A) or from −110 mV to 0 mV (B–E) in increments of 10 mV to produce each set of current recordings.

were used to dissociate this connective tissue from the axons (see Materials and methods) prior to voltage-clamp and pharmacological experiments, so that the chemicals could diffuse unimpeded, and there was clearly no barrier to diffusion because TTX rapidly blocks Na^+ currents in scorpion nerve fibres. Thus, the lack of effect of the polypeptides contained in crude *A. australis* venom demonstrates that they are unable to affect the TTX-sensitive Na^+ channels of the scorpion *A. australis*.

Comparison between crayfish and scorpion ion channels

In crayfish, the α-toxins of *A. australis* venom (AaH I and AaH II) slow the inactivation kinetics of the Na^+ current in crayfish (Rathmayer *et al.* 1977; Romey *et al.* 1976; Fig. 6A). The present study demonstrates that both the crude venom of *A. australis* (50 μg l^{-1}, Fig. 5A) and AaH II (100 nmol l^{-1}, Fig. 5C) were totally without effect on scorpion nerve fibres. These findings suggest that the voltage-sensitive Na^+ channels of these two arthropod species display some pharmacological differences. In contrast, our results show that scorpion and crayfish K^+ currents have similar pharmacological properties, suggesting that scorpion and crayfish may have similar K^+ channels.

Na^+ channels

The concept of specificity in the activity of scorpion toxins against animals of various phyla has led to the definition of ‘anti-mammal’, ‘anti-insect’ and ‘anti-crustacean’ toxins. The difference in the effects of these diverse toxins reflects structural differences between their targets. Such differences, however, result from variations in the affinities of the toxins for their receptors in different species or for different tissues

within an individual of a species. AaH I, an anti-mammal α-toxin (K_d=100 nmol l^{-1} in rat brain synaptosomes; Romey *et al.* 1976), also acts on voltage-sensitive Na^+ channels of crustacean and squid (K_d=250 nmol l^{-1} for crayfish, K_d=700 nmol l^{-1} for lobster; Romey *et al.* 1975). Similar observations were made with the potent toxin AaH II (Rathmayer *et al.* 1977). These results are consistent with the high level of sequence similarity found between the Na^+ channels of the various species. In contrast, the anti-insect toxin AaH IT1 is thought to be the most specific toxin for insects (De Dianous *et al.* 1987). We found that AaH IT1 had no effect on scorpion nerve fibres. AaH II, which does not affect insects (e.g. cockroach; D'Ajello *et al.* 1972), also had no effect on the action potential of the nerve cord of scorpion. The lack of effect of the *A. australis* venom on scorpion nerve fibres shows that the anti-crustacean toxin it contains (Zlotkin *et al.* 1972) is inactive against scorpion voltage-sensitive Na^+ channel, whereas it has a strong effect on crustacean voltage-sensitive Na^+ channels (Rathmayer *et al.* 1977).

K^+ channels

In contrast to voltage-sensitive Na^+ channels, no pharmacological differences were detected in the behaviour of K^+ channels in scorpion and crayfish. In both cases, there was no blockage of K^+ channels in either muscle or nerve fibres. Crayfish and scorpion K^+ channels are insensitive to crude venoms even at very high concentrations (*A. australis* venom, 110 μg ml^{-1} in scorpion and 180 μg ml^{-1} in crayfish; *T. serrulatus* venom, 30 μg ml^{-1} in scorpion). *A. australis* venom blocks 100 % of mollusc Ca^{2+}-activated K^+ channels at a concentration of 50 μg ml^{-1} (M. Crest, personal communication).

All the scorpion toxins tested had affinities in the nanomolar or picomolar range. For example, KTX interacts with the IK_{Ca} channels of molluscs with a K_d of 20 nmol l^{-1} (Crest *et al.* 1992). Kaliotoxin is a specific blocker of voltage-gated K^+ channels of mammals (Kv1.1, K_d=1.8 nmol l^{-1}; Kv1.3, K_d=200 pmol l^{-1}; M. Crest, personal communication), but was inactive on the Kv channel of scorpions and crayfish even at high concentrations (see Table 1). These findings suggest that the Kv currents recorded in scorpion and crayfish muscle fibres may be due to Kv channels that differ from the Kv channels present in rat skeletal and cardiac muscle (Matsubara *et al.* 1991). Charybdotoxin was inactive on Kv channels from crayfish muscle (200 nmol l^{-1}) as reported by Araque and Buño (1995) and on Kv channels from scorpion muscle fibres (10 μmol l^{-1}). It has also been shown to have no effect in *Drosophila melanogaster*, as demonstrated *in vivo* in a nerve–muscle preparation (Zagotta *et al.* 1989). Thus, it seems that, in invertebrates, native Kv channels are insensitive to scorpion toxins. They also appear to be insensitive to DTX, which is a highly specific ligand of vertebrate Kv1.1 and Kv1.2 channels (Stühmer *et al.* 1989; Rehm, 1991), but has absolutely no effect on scorpion or crayfish Kv channels. Therefore, *in vivo*, invertebrate Kv channels have pharmacological properties that are consistently different from those of vertebrates.

We detected no inward K^+ currents in scorpion muscle fibres, whereas such currents were detected in crayfish muscle fibres. Further work is required because the physiological function of these inward K^+ currents is unclear. Our results with BK_{Ca} channels in crayfish are in conflict with other observations. Although we detected no BK_{Ca} currents in scorpion preparations, as described in the muscle fibres of the scorpion *Centruroides sculpturatus* (Gilly and Scheuer, 1993), small BK_{Ca} currents were recorded in crayfish muscle fibres in Ca^{2+}-free solutions. High concentrations of ChTX (400 nmol l^{-1}) have been shown to block BK_{Ca} channels in crayfish opener muscle fibres (Araque and Buño, 1995) and in the presynaptic terminals at the neuromuscular junction of crayfish (Sivaramakrishnan *et al.* 1991). However, in the present study, ChTX had no significant effect on BK_{Ca} channels at concentrations up to 200 nmol l^{-1} in crayfish muscle. This lack of effect may be due to the use of a low concentration of ChTX in these experiments, but this seems unlikely since other authors have reported that IbTX (the most specific and potent BK_{Ca} blocker with a K_d of 1.2–1.7 nmol l^{-1}) is inactive on BK_{Ca} channels from crayfish muscle at concentrations up to 1 μmol l^{-1} (Blundon *et al.* 1995). These data indicate that crayfish BK_{Ca} channels differ pharmacologically and structurally from those of vertebrates. The structure of the crayfish Kv channel is not yet known; to date, the only work on this subject concerns the gene encoding a lobster Kv channel (a *shal*-related channel), which has been isolated and expressed in oocytes (Baro *et al.* 1996).

In conclusion, the muscular and nervous systems of scorpions are not affected by the molecules contained in scorpion venom. Mutation of essential amino acid residues implicated in the interaction between toxins and the receptor may be the mechanism underlying this resistance (Gross *et al.* 1994). This would be consistent with other examples in the animal kingdom, such as the mutation of a few amino acid residues in the binding site of the mongoose and snake acetylcholine receptors for α-bungarotoxin and other snake α-neurotoxins (Barchan *et al.* 1992). Similarly, selection pressure may be responsible for the resistance of the scorpion to its toxic secretions. Work is in progress to isolate the genes coding for the K^+ and Na^+ channels of crayfish.

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