Br. J. Pharmac. (1983), **78**, 529–532

Effects of the venom of the Brazilian scorpion (*Tityus serrulatus*) on the compound action potential of the rabbit vagus nerve fibres

Francisco Riccioppo Neto

Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, 14100 Ribeirão Preto, São Paulo, Brazil

1 The effects of the venom of the scorpion (*Tityus serrulatus*) on nerve fibres of the rabbit cervical vagus were studied by the single sucrose-gap technique. Scorpion venom (1 μg/ml) increased irreversibly the duration of the B component of the compound action potential of the vagus nerves, leaving the C component with its normal configuration. Tetrodotoxin (200 n M) supressed the prolongation of the action potential duration in venom-treated B fibres.

2 At the same concentration (1 μg/ml), scorpion venom reduced the amplitude and the rate constant of decay of the hyperpolarization produced by tetanic stimulation of non-myelinated nerve fibres.

3 A lower concentration (0.2 μg/ml) blocked completely the hyperpolarization of the potassium-activated response. After washing, the potassium-activated response partially recovered its amplitude but there was a significant increase in the time constant of the decay of hyperpolarization.

4 It is suggested that scorpion venom may modify the sodium pumping mechanism within fibres as well as affecting the passive and active sodium permeability systems.

Introduction

Scorpion venoms induce a prolongation of the action potential in most nerve fibres by inhibiting the inactivation mechanism of the sodium channel (Narahashi, Shapiro, Deguchi, Scuka & Wang, 1972; Gillespie & Meves, 1980). It has been proposed, although never directly proved, that a similar prolongation of the action potential of the non-myelinated portion of nerve terminals, at presynaptic level, could also account for the great increase in the transmitter release which follows scorpion venom application (Warnick, Albuquerque & Diniz, 1976; Adler-Graschinsky & Langer, 1978). In the present work the effects of the venom of the scorpion *Tityus serrulatus* on the compound action potential and sodium pumping activity of the rabbit vagus nerve are described, and an additional mechanism is suggested to explain the increased neurotransmitter release by the venom.

Methods

Segments of about 7 cm of cervical vagus nerves were obtained from adult rabbits (3–4 kg) killed by a blow on the head. The desheathed nerve was mounted in a modified sucrose-gap apparatus (Riccioppo Neto, 1978). In order to avoid the short-circuiting effect of chloride ions during hyperpolarization, the nerves were bathed in chloride-free Locke solution containing (mM): Na-isethionate 154, K_2SO_4 2.8, $CaSO_4$ 5, Tris 8 brought to pH 7.6 with H_2SO_4, and glucose 5. Preliminary experiments showed that the effects of scorpion venom on the action potentials of nerve fibres bathed in normal Locke solution were practically the same after exclusion of the chloride ions. Compound action potentials were elicited by means of supramaximal square wave pulses of 0.5 ms duration (0.05 ms for myelinated nerve fibres). The procedures for recording changes in resting and action potentials were described previously (Riccioppo Neto, 1980). Post-tetanic hyperpolarizations were obtained using a period of 5 s of stimulation at a rate of 30 Hz. Potassium-activated responses were obtained following the method described by Den Hertog (1973): the nerves were first superfused with K^+-free Locke solution for 15 min, followed by the readmission of K^+ (5.6 mM). The time constants of decay of the hyperpolarization were obtained from semilogarithmic plots of the amplitude of the recov-

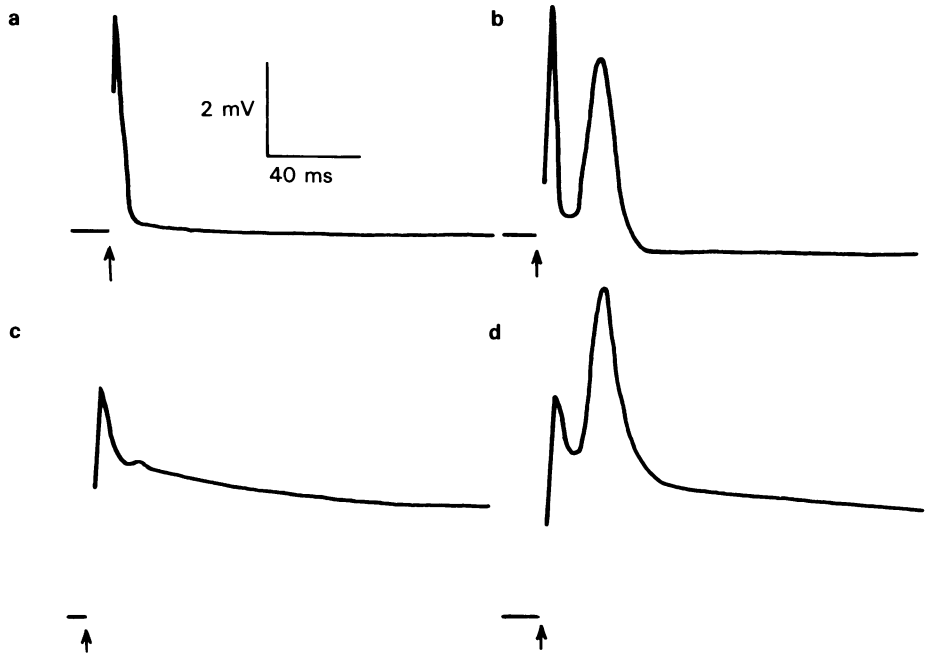

Figure 1 Effects of the scorpion venom (1 μg/ml) on the compound action potential of the rabbit vagus nerve. The horizontal bar at the beginning of each trace is the prestimulation baseline. Stimulus was applied at the arrows. (a) Control: B component obtained with a supramaximal stimulation pulse of 0.05 ms duration; (b) control: B and C components obtained with pulse of 0.5 ms duration. (c and d): 20 min during venom superfusion; (c) B component; (d) B and C components. Note that a C component of normal configuration superimposes on a prolonged B component in venom-treated fibres.

ery phase vs. time (see Figure 2). The experiments were carried out at room temperature (20 °C). Whenever possible means ± s.e. mean are given. Desiccated venom, obtained from the scorpion *Tityus serrulatus* by the method of Zlotkin & Shulov (1969), was first dissolved in distilled water to make up a stock solution of 1 mg/ml and diluted with bathing solution to prepare test solutions. Tetrodotoxin (TTX) was purchased from Sigma.

Results

The application of scorpion venom (1 μg/ml, during 30 min) to six nerves, induced an irreversible prolongation of the duration of the B component of the compound action potential. However, the C component, corresponding to non-myelinated fibres, maintained its normal configuration (Figure 1). There were no significant alterations in the resting potential or in the amplitude of the action potentials. The addition of TTX in a partially blocking concentration (200 nM; $n = 3$) suppressed the prolongation of the B component of the action potential leaving only a spike of reduced amplitude and nearly normal configuration. In five experiments, scorpion venom (1 μg/ml) decreased the amplitude of the post-tetanic hyperpolarization from 5.88 ± 1.03 mV to 3.25 ± 1.03 mV ($P < 0.01$; paired Student's t test). On these nerves, the time constant for the decay of hyperpolarization increased from 0.91 ± 0.09 min (control) to 1.41 ± 0.06 min ($P < 0.01$). These effects were partially reversed on washing. In a concentration as low as 0.2 μg/ml perfused for 20 min, the venom completely blocked the potassium-activated response in six other nerves. After 60–90 min washing in venom-free solution there was some recovery in the amplitude of the potassium-activated response. At this time, the time constant of the recovery of the hyperpolarization was greatly (110 ± 22%) increased (Figure 2).

Discussion

Scorpion venom has been shown to prolong irreversibly the falling phase of the B component of the compound action potential of the rabbit vagus nerve. The C component, corresponding to the non-myelinated fibres, was left unaffected. This is in

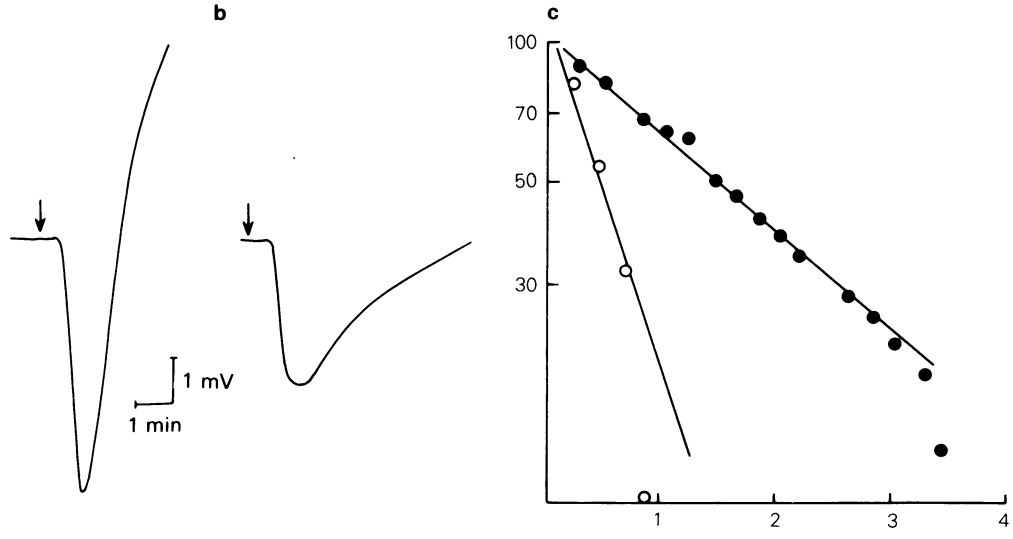

Figure 2 The potassium-activated response (PAR) of the rabbit vagus nerve recorded before (a) and (b) 90 min after washing out scorpion venom (0.2 μg/ml) applied for 20 min. Potassium was readmitted at the arrow. (c) Semi-logarithmic plot of the time course of the decay of the PAR shown in (a) (open circles) and (b) (closed circles); abscissa scale: time (min) measured from the peak of the hyperpolarization; ordinate scale: % amplitude of the decay of the hyperpolarization.

contrast to the action of veratrine, which consistently prolonged the falling phase of the action potential of C fibres in vagus nerves in concentrations (0.25–0.5 μg/ml) that did not affect the B fibres (Riccioppo Neto, unpublished results). The effects of the scorpion venom on the post-tetanic hyperpolarization and on the potassium-activated response of non-myelinated nerve fibres are highly suggestive of an inhibitory action on the sodium pumping mechanism. These effects were observed at concentrations of venom in which the resting potential and the action potential were practically unaffected. It seems possible that they are reflecting a decrease in pumping activity as well as a lesser amount of sodium extruded (Rang & Ritchie, 1968). Since scorpion venom had no effect on the Na-K-ATPase enzyme isolated from rat brain homogenates (Gomez, Diniz & Barbosa, 1975; Larsen & Riccioppo Neto, unpublished), it might be exerting actions of the type described for metabolic inhibitors such as 2-4-dinitrophenol and cyanide (Den Hertog & Ritchie, 1969). Birks & Cohen (1968a) have shown that sodium pump inhibitors can induce an increase in the transmitter release in frog isolated skeletal muscle and that the effects are probably due to the accumulation of intracellular sodium (Birks & Cohen, 1968b). The inhibition of the Na^+-pump could therefore be an alternative mechanism through which scorpion venom increases neurotransmitter release. The crude venom used certainly is a mixture of many active substances and further experiments with different chemical fractions of this toxin are now under way.

I thank Dr A. Antonio for many discussions and Dr I. Melito for kindly supplying scorpion venom. This investigation was supported in part by a grant from the Fundação de Amparo à Pesquisa do Estado de São Paulo–FAPESP (Biol. 80/0998-6).

References

ADLER-GRASCHINSKY, E. & LANGER, S.Z. (1978). Mechanism of the enhancement in transmitter release from central and peripheral noradrenergic nerve terminals induced by the purified scorpion venom, Tityustoxin. *Naunyn-Schmiedebergs Arch. Pharmac.*, **303**, 243–249.

BIRKS, R.I. & COHEN, M.W. (1968a). The action of sodium pump inhibitors on neuromuscular transmission. *Proc. R. Soc. B.*, **170**, 381–400.

BIRKS, R.I. & COHEN, M.W. (1968b). The influence of internal sodium on the behaviour of motor nerve endings. *Proc. R. Soc. B*, **170**, 401–421.

DEN HERTOG, A. (1973). Some further observations on the electrogenic sodium pump in non-myelinated nerve fibres. *J. Physiol.*, **231**, 493–509.

DEN HERTOG, A. & RITCHIE, J.M. (1969). A comparison of

the effect of temperature, metabolic inhibitors and of ouabain on the electrogenic component of the sodium pump in mammalian non-myelinated nerve fibres. *J. Physiol.*, **204,** 523–538.

GILLESPIE, J.I. & MEVES, H. (1980). The effect of scorpion venoms on the sodium currents of the squid giant axons. *J. Physiol.*, **308,** 479–499.

GOMEZ, M.V., DINIZ, C.R. & BARBOSA, T.S. (1975). A comparison of the effects of scorpion venom tityustoxin and ouabaïn on the release of acetylcholine from incubated slices of rat brain. *J. Neurochem.*, **24,** 331–336.

NARAHASHI, T., SHAPIRO, B.I., DEGUCHI, T., SCUKA, M. & WANG, C.W. (1972). Effects of scorpion venom on squid axon membranes. *Am. J. Physiol.*, **222,** 850–857.

RANG, H.P. & RITCHIE, J.M. (1968). On the electrogenic sodium pump in mammalian non-myelinated nerve fibres and its activation by various external cations. *J. Physiol.*, **196,** 183–221.

RICCIOPPO NETO, F. (1978). The depolarizing action of 5-HT on mammalian non-myelinated nerve fibres. *Eur. J. Pharmac.*, **49,** 351–356.

RICCIOPPO NETO, F. (1980). Further studies on the actions of salicylates on nerve membranes. *Eur. J. Pharmac.*, **68,** 155–162.

WARNICK, J.E., ALBUQUERQUE, E.X. & DINIZ, C.R. (1976). Electrophysiological observations on the action of the purified scorpion venom, Tityustoxin, on nerve and skeletal muscle of the rat. *J. Pharmac. exp. Ther.*, **198,** 155–167.

ZLOTKIN, E. & SHULOV, A.S. (1969). A simple device for collecting scorpion venom. *Toxicon*, **7,** 331–332.

(*Received July 20, 1982.*
Revised October 20, 1982.)