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THE EFFECT OF SCORPION VENOM ON SINGLE MYELINATED NERVE FIBRES OF THE FROG

BY

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Initial investigations on the action of scorpion venom on isolated skeletal muscle and desheathed bundles of nerve fibres were made some time ago (Adam & Weiss, 1958, 1959a, 1959b).

The results suggested that the active principle of scorpion venom exerts a direct effect on excitable membranes. The present investigation on single Ranvier nodes of isolated nerve fibres was undertaken in order to examine the electrophysiological effects of the venom more fully.

METHODS

Single nerve fibres were isolated from either the motor branch of N. tibialis to the gastrocnemius muscle or from the ramus cutaneous cruris posterior of *Rana esculenta*. The fibre was mounted in an air-gap arrangement which allowed one node to be superfused continuously (Stämpfli, 1956) with Ringer or test solutions. The inter-nodes on either side bridged short air-gaps, the neighbouring nodes being placed in side pools containing 0.2% cocaine-Ringer solution to avoid activity of these nodes. A special stop-cock situated near the superfused node allowed the rinsing fluid to be changed in less than one second.

Changes of resting and action potentials were led off by means of calomel-electrodes between the central node and one of its neighbours; they were measured by a differential amplifier of high input impedance in a negative feedback arrangement (Schmidt & Stämpfli, 1966). This method allows the measurement of membrane potential changes to about 90% of their true values. The output potential of the amplifier was recorded on an oscilloscope (Tektronix Type 502 A), and simultaneously by a Varian ink-writer (G 14). In addition the output signal was differentiated by a passive RC-network with a time constant of 10 μsec.

For stimulation of the central node Tektronix type 161 units were used; the pulses were applied between the two nodes which were separated by the second air-gap.

In the voltage clamp experiments the amplifier input and output were connected to the side pools. The membrane potential of the central node was changed to the desired values by the application, between this node and earth, of a voltage delivered by a function generator (Exact Electronics Inc. Hillsboro, Oregon). Clamp potential and membrane current were measured on the oscilloscope using X-Y-recording. The experiments were performed at room temperature (18 to 24° C).

Venom was obtained from *Leiurus quinquestriatus* (H. & E.) by electrical stimulation of the telson, suspended in water, centrifuged (3,000 g, 10 min), and the supernatant freeze-dried. Bulked material, obtained in this way, was dissolved in water, cold acetone added (to 80% v/v) and left

overnight at −15° C. The resulting precipitate was washed twice with cold, dry acetone, and air-dried (cf. Miranda, Rochat & Lissitsky, 1960).

Such material contains two basic proteins of low molecular weight, similar to those described in the venom of *Androctonus australis* and *Buthus occitanus* (toxins I and II, Miranda, *et al.*, 1964a, 1964b) but, for the purpose of this work, further fractionation was not carried out.

Ringer solution of the following composition (mM/l.) was used as rinsing fluid: NaCl 110.5; $NaHCO_3$ 2.4; KCl 2.5; $CaCl_2$ 1.8. In the low-sodium solutions NaCl was substituted on a molar basis by choline chloride (Merck). The extra calcium chloride in the high calcium solutions was simply added to the normal Ringer solution.

The tetraethylammonium chloride used in some experiments was obtained from Fluka, Switzerland; the 5-hydroxytryptamine was supplied by Merck, Germany.

RESULTS

General description of the effects of scorpion venom on the Ranvier node

Ranvier nodes were stimulated electrically with a frequency of one per sec. Addition of venom, in the lowest effective concentration (5×10^{-8} g/ml.), to Ringer solution produced a progressive prolongation of the action potential. Resting potential, spike potential and maximum rate of rise of the action potential remained initially unchanged. The delay between the beginning of exposure to the venom and the first sign of an effect on the electrical properties of the nodal membrane depended strongly on the

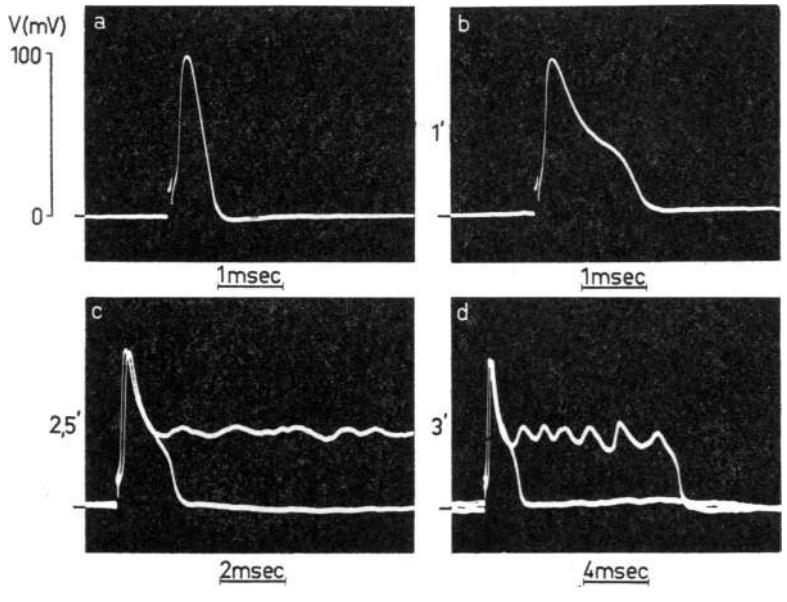

Fig. 1. Action potentials of a sensory fibre in normal Ringer solution (*a*); and (*b*) 1 min, (*c*) 2.5 min and (*d*) 3 min after addition of 5×10^{-7} g scorpion venom/ml. The node has been stimulated at a rate of 1/sec; *c* and *d* each show two action potentials elicited by successive stimuli. One action potential in each Fig. shows a plateau with superimposed oscillations of 10 to 20 mV amplitude. In *c* and *d* the resting potential shows small irregular oscillations. Ordinate: amplitude of the action potential; V = O corresponds to the normal resting potential. t = 20° C.

concentration of venom but varied to some extent from fibre to fibre. However, there was no significant difference in this lag period between motor and sensory fibres.

At a higher concentration of the venom (5×10^{-7}) the action potential developed a plateau after about 2 min of exposure (Fig. 1). This plateau often showed irregular oscillations of 10–20 mV amplitude at a frequency of about 200/sec. The duration of the plateau at this stage varied greatly, as is demonstrated in Figs. 1,*c* and *d*; repolarization sometimes occurred without any conspicuous plateau. The maximum slope of the upstroke, the spike potential and the beginning of the repolarization phase remained practically unchanged, whereas the resting membrane depolarized slightly; small, slow and irregular oscillations of resting potential developed. Continuous exposure to the venom in a concentration $\geqslant 5 \times 10^{-7}$ led to spontaneous activity accompanied by a depolarization of the membrane. The fibres always remained electrically excitable unless the depolarization reached values of about 30 mV. The described effects of scorpion venom were never fully reversible; however, the effects of a short exposure to low concentrations of the venom (5×10^{-8}–10^{-7}) could be reversed

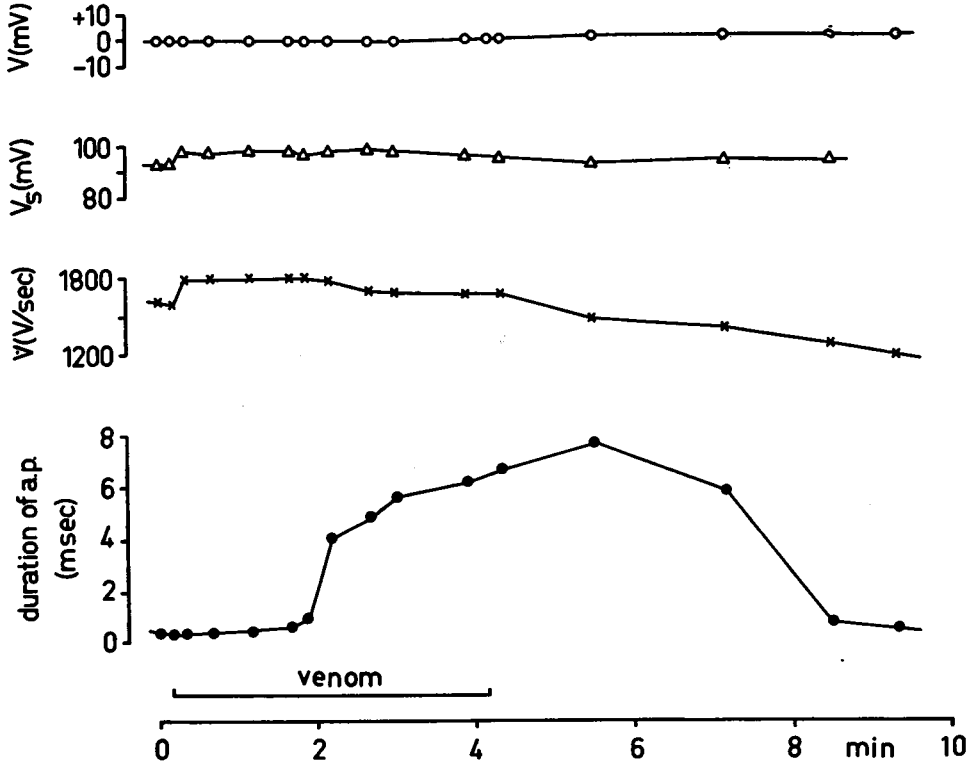

Fig. 2. Resting potential change (V), spike potential (V_s), maximum rate of rise ($\dot{V}$) and duration of the action potential (a.p.) of a sensory fibre before, during and after exposure to 10^{-7} g venom/ml. The exposure time was 4 min (horizontal line). The duration of the action potential was measured as the time between maximum rate of rise and maximum rate of fall of the action potential. The duration before application of the venom was 0.4 msec; t=24° C.

to some extent by prolonged rinsing in Ringer solution. Fig. 2 demonstrates the degree of reversibility of the venom effect on the action and resting potentials. In this experiment the resting and spike potentials remained virtually unchanged, while the slope of the upstroke, after a small initial increase, fell continuously without any sign of reversibility. Only the duration of the action potential returned to approximately normal values after a period of washing with Ringer solution.

5-Hydroxytryptamine occurs in high concentration in *L. quinquestriatus* venom (2–4 mg/g dried crude venom; Adam & Weiss, 1958). Applications of 5-hydroxytryptamine, in concentrations up to 10^{-6} g/ml., to Ranvier nodes did not change the resting or action potentials or produce spontaneous activity.

Influence of Na on the effect of the venom

Low sodium solutions abolished the effects of scorpion venom completely. In the experiment of Fig. 3 venom (10^{-6} g/ml.) was applied for a period of 4 min in a solution containing only 2 mM Na/l., the rest of the NaCl being substituted by choline chloride. At the end of this period the membrane was hyperpolarized by 8 mV, the resting potential being very stable. A sudden increase of the Na-concentration by rinsing the node with Ringer solution produced a slow depolarization and a short spike (not visible in Fig. 3), followed by a declining plateau of 12 sec duration. The plateau ended abruptly when a critical potential level, which corresponded roughly to the so-called repolarization threshold of normal fibres was reached. The membrane potential approached a value near V=5 mV; but the membrane was again depolarized slowly until another spontaneous discharge occurred. The frequency of repetition in the experiment of Fig. 3 increased slightly to about 5/min. In other experiments it varied between less than 1/min and 250/sec. Apparently the frequency depended on the level of depolarization. The maximum rate of spontaneous repetition was observed at depolarizations of about 10 mV. In Fig. 3,*e*, where the Na-concentration was again reduced to 2 mM/l. spontaneous activity ceased immediately, and the previous level of hyperpolarization was reached again.

The spontaneous firing of a motor fibre, which had been treated with 5×10^{-7} g venom/ml. is shown in Fig. 4. Within 4 min after the first contact with the venom spontaneous action potentials occurred at a mean rate of 2/min; subsequently a slow depolarization developed, which eventually, in the course of the ensuing 14 min, reached a value of 8 mV. Concomitant with this depolarization the frequency of spontaneous discharges increased to 8/sec. Fig. 4 was taken at this stage; each action potential was followed by a short after-hyperpolarization, which decayed slowly. Small oscillations increased slightly in amplitude until at a critical potential level the next action potential started.

The changes of resting potential brought about by the venom depended largely on the sodium concentration of the medium. Fig. 5 shows the relationship between the sodium concentration and the resting potential of 14 single fibres before and after a period of contact with venom of at least 4 min. At low sodium concentrations the hyperpolarization exceeded that before application of the venom, while at sodium concentrations above 40 mM/l. a depolarization occurred. The relationship between the amplitude of depolarization and the log of the sodium concentration had a maximum slope of 40 mV/

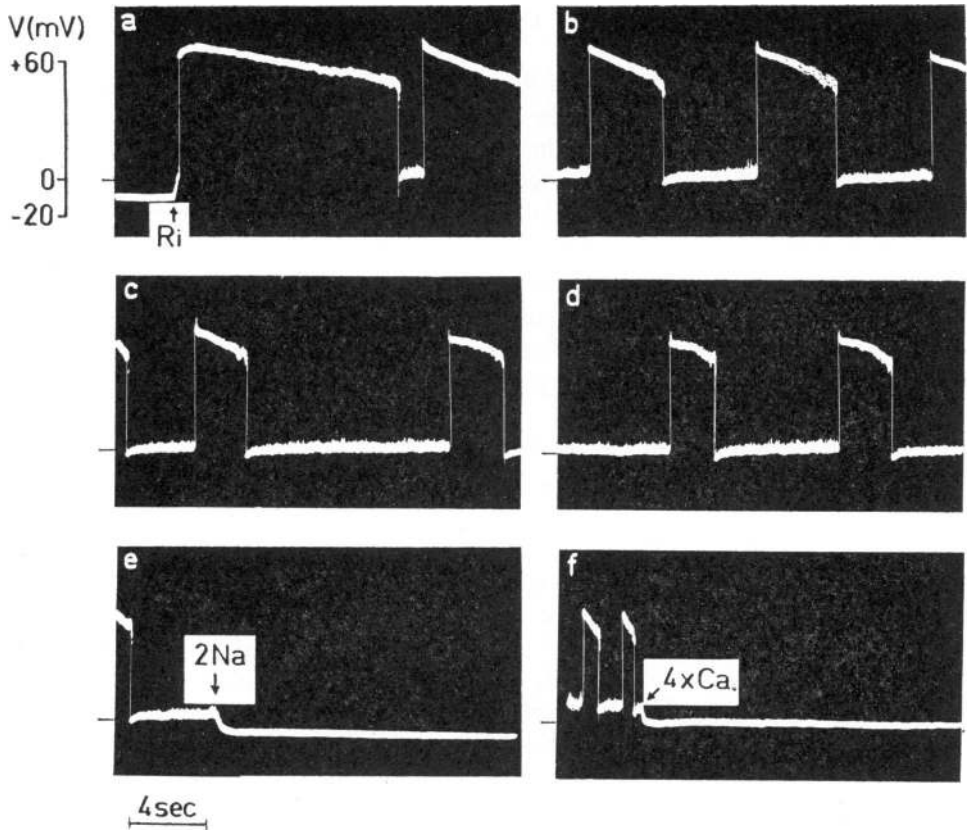

Fig. 3. Potential changes in a motor fibre which had been exposed to scorpion venom (10^{-6} g/ml.) in a solution containing only 2 mM Na/l. After 4 minutes the low sodium solution was exchanged for Ringer solution (arrow Ri); following a slow depolarization a spike was fired off, whose peak was some 30 mV higher than the beginning of the plateau in Fig. 3,*a*. Figs. *a* to *f* show spontaneous activity during successive sweeps; the time mark 4 sec (below *e*) applies to all Figs. In *e* the Na-concentration was suddenly reduced; the node repolarized to the original potential level and spontaneous activity was stopped. In *f* normal Ringer solution has been exchanged for Ringer solution containing 7.2 mM Ca/l. (4 times normal). The repolarization was somewhat less than in *e*, but spontaneous activity ceased immediately.

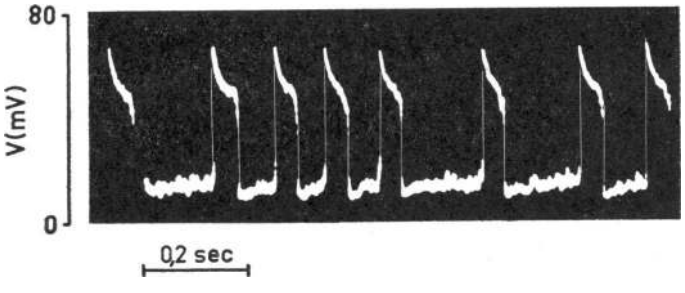

Fig. 4. Spontaneous activity of a motor fibre superfused with Ringer solution containing 5×10^{-7} g venom/ml. The photograph has been taken 18 minutes after the beginning of exposure to the venom. Following each action potential the membrane potential reaches a value of about V=8 mV; then a slow depolarization occurs, on which small oscillations are superimposed, until at a critical potential level the next action potential appears. t=20° C.

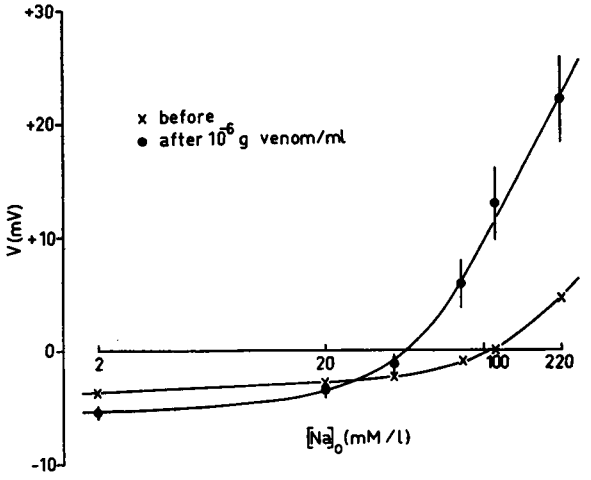

Fig. 5 ; Relationship between Na-concentration of the medium and membrane potential before and after exposure to scorpion venum (10^{-6} g/ml.). Ordinate, change of membrane potential in mV ; depolarization positive. V=O is the normal resting potential before application of the venom. Mean values and standard errors of the means of 14 (9 motor and 5 sensory) fibres. The standard errors of the means in venom-free solutions are smaller than the symbols. Abscissa, sodium concentration of the outside medium in mM/l. on logarithmic scale. Each fibre has first been rinsed with venom-free solutions of different Na-concentrations for about 1 min. Then the fibre was treated with 10^{-6} g venom/ml. in 2 mM Na-solution ; after at least 4 min exposure, the Na-concentration was stepwise increased to the same values as before. The depolarization always showed an overshoot, but after 1–4 min a constant value was attained. These steady state values have been used to calculate the points in Fig. 5. Between each Na-concentration the fibre was superfused with 2 mM Na-solution for several minutes. Fig. 5 shows that the venom increases the hyperpolarization in Na-poor solutions and produces a depolarization with Na-concentrations larger than 40 mM/l. The curves were drawn by eye.

10-fold concentration change, thus indicating a marked increase of the sodium permeability of the resting membrane.

The amplitude of depolarization produced by the venom increased considerably (by 20 to 30 mV) when tetraethylammonium-chloride (TEA) in a concentration of 5 mM/l. was added to the medium.

Substitution of Na by Li did not alter the response of the nodal membrane to scorpion venom.

Influence of Ca-ions and hyperpolarizing currents

Calcium has been found previously to be a potent inhibitor of scorpion venom (Adam & Weiss, 1959a). Fig. 6 demonstrates that addition of Ca^{2+} to the rinsing fluid stops spontaneous activity of the poisoned fibre and shortens the action potential considerably ; and Fig. 3 shows that the action of Ca^{2+} on the nodal membrane is an immediate one.

Calcium consistently reduced the amplitude of depolarization brought about by the venom in Na-containing solutions. Eleven fibres, which had been depolarized by venom

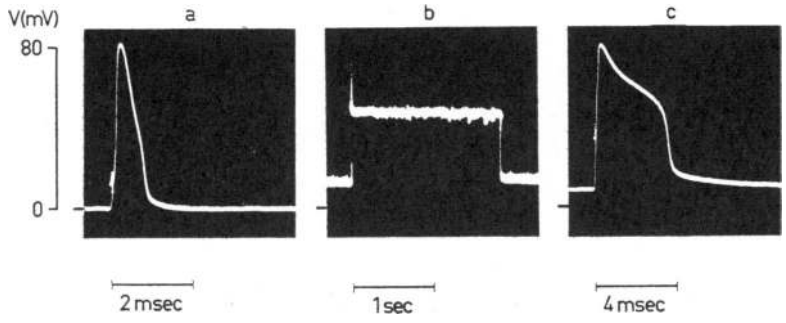

Fig. 6. Effect of increasing the Ca-concentration of the medium on the duration of the action potential. Fig. *a* shows the action potential of a motor fibre elicited by a 50 μsec pulse in normal Ringer solution. After 16 minutes exposure to Ringer solution containing 10^{-7} g venom/ml. the fibre had been depolarized by 13 mV and became spontaneously active at a rate of about 4/min. Fig. *b* shows an action potential of about 2 sec duration taken at that time. Resting potential and plateau are superimposed by small oscillations. After increasing the Ca-concentration from 1.8 to 7.2 mM/l. the fibre partially repolarized; the resting membrane potential remained stable and spontaneous activity ceased completely. Application of a short stimulus to the fibre was followed by the action potential shown in *c*; t=20.5° C.

to V=6–38 (mean 18) mV, were exposed to Ringer containing 7.2 mM Ca^{2+}/l.; in 4 fibres, the normal resting potential was restored; in the remaining 7 fibres, the depolarization was reduced to a mean value of 6 mV.

A similar effect to that of raising the Ca-concentration could be observed while feeding a hyperpolarizing current to the node. Fibres which had been depolarized by more than 30 mV and showing only small and irregular oscillations of the membrane potential could be made to fire spontaneously by moderate anodal polarization, whilst a stronger anodal polarization stopped spontaneous activity again. A short anodal pulse applied during the plateau of a prolonged action potential abolished the action potential in an all-or-none manner. This phenomenon (anodal abolition) has been described in detail by Tasaki (1956) and by Lüttgau (1956) in normal frog nerve fibres.

Delayed rectification in the poisoned nodal membrane

Since the action potential can be prolonged to some extent by a reduction of the potassium permeability (Frankenhaeuser & Huxley, 1964), experiments were carried out in order to examine the possible influence of scorpion venom on the delayed rectification. Rectangular current pulses of various intensities and constant duration (100 msec) were applied to the node before and after a period of poisoning (4 min) with 5×10^{-7} g venom/ml.; the sodium content of the rinsing solution was reduced to 2 mM/l. As has already been described by several workers (Schoepfle, 1959; Meyes, 1963; Schmidt & Stämpfli, 1966), the membrane potential reaches a maximum value immediately after the onset of the current pulse and then declines towards a constant final value. This behaviour of the membrane is due to a delayed increase of the potassium permeability upon depolarization. In two fibres the delayed rectification developed more slowly after

poisoning; six fibres showed no difference from normal fibres as regards time course and amount of delayed rectification.

Current-voltage relation of the nodal membrane under the influence of scorpion venom

The results demonstrated in Fig. 5 suggest a large increase of the sodium permeability of the resting membrane under the influence of venom. This assumption is supported by the results of some preliminary voltage-clamp experiments. The membrane potential of a poisoned node (10^{-6} g venom/ml.) was changed under voltage-clamp conditions at a constant rate of 8 mV/sec in either direction (as indicated by the arrows in Fig. 7). Fig. 7,*a* shows the relation between membrane current and membrane potential in a low

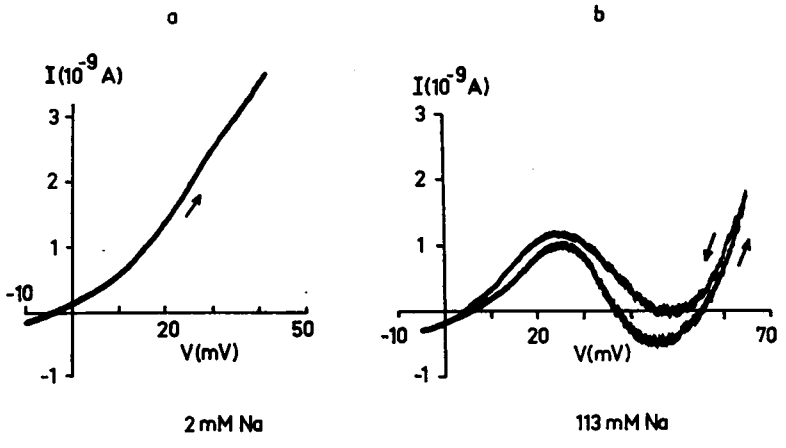

Fig. 7. Current-voltage relation of a motor fibre in 2 mM Na-solution (*a*) and in normal Ringer solution (*b*), after 5 min poisoning with 10^{-6} g venom/ml. Ordinates, membrane current in units of 10^{-9} A; Abscissa, change of membrane potential in mV; V=O is the resting potential in normal Ringer solution before application of the venom; depolarization positive. The membrane potential was changed linearly at a rate of 8 mV/sec; arrows indicate direction of potential change. Due to lack of sodium ions, the nodal membrane was hyperpolarized by about 5 mV in *a*; the current-voltage curve is similar to that of an unpoisoned node superfused with low or normal sodium solutions. In the presence of normal sodium (*b*) the membrane was depolarized by 5 mV due to the increased permeability of the resting membrane to sodium ions. The current-voltage curve obtained by changing the membrane potential from V=−5 to +65 mV three times intersects the zero current line and shows a region of net inward current between V=35 and 55 mV; the curve recorded in the opposite direction only touches the zero current line near V=50 mV. The N-shape of the two curves is due to an inward current of sodium ions since with replacement of sodium by choline ions (Fig. 7,*a*) there is no sign of inward current.

sodium solution. The curve does not differ significantly from curves obtained from an unpoisoned node in either low or normal sodium solutions; it shows the well-known rectification of the nodal membrane upon depolarization, which is due to an increase of the potassium-permeability. In the present experiment the current-voltage curve was recorded in one direction only; other experiments, however, showed that there are but negligible differences between the curves obtained in low sodium solutions by changing the membrane potential in opposite directions.

However, in solutions containing high concentrations of sodium the current-voltage curve is N-shaped between V=20 and V=60 mV (Fig. 7,*b*). The demonstration of a region with negative resistance under these conditions can only be explained by a large inward current of sodium ions due to an increased permeability of the membrane. When the membrane potential was changed in the direction of depolarization the sodium current became even larger than the outward current (mainly potassium ions) associated with depolarization; the curve therefore intersects the zero current line 3 times and shows a region of net inward current between V=35 and 55 mV.

The increase of the sodium permeability apparently persists for many seconds since the current-voltage curve obtained in the opposite direction (changing the membrane potential from depolarization to hyperpolarization) is still N-shaped; but it has moved in the direction of positive current and only touches the zero current line near V=50 mV. In this experiment the rate of potential change was such that the potenial value V=50 mV was reached again after about 4 sec. During this time the sodium permeability must have been inactivated to some extent; the sodium current was therefore reduced and the net current no longer inward.

The fact that the current-voltage curves in normal and low sodium solutions have similar slopes (dI/dV) near V=O indicates that in this potential range the sodium permeability is small.

DISCUSSION

The first sign of an effect of scorpion venom on the nodal membrane is a progressive prolongation of the action potential. It is noteworthy that at this stage the maximum rate of rise, the amplitude and the initial phase of repolarization of the action potential are unchanged. This observation suggests that the fast increase of the sodium permeability which is responsible for the rising phase of the action potential remains largely unaffected.

The falling phase of the action potential is determined by a complex change of the sodium and potassium permeabilities of the membrane (Hodgkin & Huxley, 1952; Frankenhaeuser & Huxley, 1964). A prolongation of the action potential can therefore be due to a delayed decrease (inactivation) of the sodium permeability or a reduction or a delayed increase of the potassium permeability. Since the venom has no effect on the delayed rectification, it can be concluded that the potassium permeability is virtually unchanged.

The assumption of a delayed inactivation of the sodium permeability is supported by the results of preliminary voltage-clamp experiments. It is well known that a node in normal Ringer solution, submitted to stepwise depolarization, shows for a short period (<2 msec) an initial transient ionic current which is carried by sodium ions (Dodge & Frankenhaeuser 1959). The relation between the peak values of this transient sodium current and the membrane potential is N-shaped. With a node poisoned by scorpion venom such a current-voltage curve is demonstrable for many seconds. An N-shaped current-voltage curve can be recorded even if the membrane potential is changed at a constant rate of only several mV/sec. Thus in Fig. 7 the direction of membrane current changed from outward to inward in the potential range between V=35 and 55 mV.

However, this result could only be observed provided the sodium concentration of the medium was sufficiently high and the clamp-potential changed from the normal resting level in the direction of depolarization. When moving the clamp-potential in the opposite direction the curve still remained N-shaped, but the inward component of membrane current became smaller or disappeared. This is easily explained by the assumption that the sodium permeability was partially inactivated during the previous depolarization. It can therefore be assumed that the inactivation process under the influence of scorpion venom has a time constant of the order of seconds ; since the action potential of the poisoned membrane sometimes lasts many seconds, the rate of inactivation of the sodium permeability is clearly of the right order of magnitude to account for the prolongation of the action potential.

This explanation of the prolonged action potential is supported by the experiments on anodal abolition. An all-or-none repolarization can only be produced provided the current-voltage relation during the plateau of the action potential contains a region with negative resistance.

The instability of the membrane potential during the plateau phase seems to be reflected by the instability of the current-voltage curve at potential levels greater than $V=20$ mV. This instability is obviously due to spontaneously occurring changes of the sodium permeability since it is not present in low sodium solutions. Similar oscillations have been observed near the normal resting potential level ; if accompanied by a moderate depolarization of the membrane they are responsible for the generation of impulses.

The fact that scorpion venom very markedly affects the sodium permeability of the nodal membrane is further demonstrated by its influence on the relationship between membrane potential and sodium concentration of the medium. In normal fibres lack of sodium hyperpolarizes the membrane by a few mV, whereas doubling the sodium concentration produces a depolarization of some 5 mV (Fig. 5). This result is in agreement with measurements of Huxley & Stämpfli (1951) on single fibres and Schmidt (1964) on bundles of nerve fibres. After poisoning, the hyperpolarization in low sodium solutions is somewhat strengthened, but there is already a depolarization with 80 mM Na/l. ; the depolarization amplitude rises with increasing sodium concentration and the slope of 40 mV per tenfold concentration change suggests that the resting membrane under the influence of scorpion venom is much more permeable to sodium ions than the unpoisoned membrane.

This increase of the sodium permeability can be partially inhibited by addition of Ca^{2+} to the medium. The membrane therefore repolarizes and spontaneous activity ceases. These results correspond with earlier observations on isolated skeletal muscle, where a surmountable antagonism between scorpion venom and Ca^{2+} has been observed (Adam & Weiss, 1959a).

Some effects of scorpion venom are similar to the effects of veratridine on the nodal membrane. Both substances produce strong depolarizations of the resting membrane in the presence of sodium ions in the medium (Straub, 1956 ; Schmidt, 1964 ; Ulbricht & Flacke, 1965). These depolarizations can be augmented considerably by addition of tetraethylammonium chloride (TEA). TEA is known to reduce the potassium perme-

ability of the nodal membrane (Schmidt, 1965; Schmidt & Stämpfli, 1966); therefore the membrane potential in the presence of TEA moves towards the sodium equilibrium potential, which is near $V = 125$ mV (Dodge & Frankenhaeuser, 1959). An increase of the Ca-concentration of the medium to only four times its normal value abolishes most effects of veratridine and scorpion venom.

There is, however, one striking dissimilarity. Veratridine produces long-lasting after-depolarizations which decay exponentially towards the resting potential level (Ulbricht & Flacke, 1965); but it has never been observed to produce action potentials with plateaus of several sec duration and oscillations. With action potentials lasting more than 10 sec, scorpion venom seems to be the most potent substance as regards its effect on the duration of the action potential. TEA prolongs the action potential of the Ranvier node at room temperature (21.5° C) about three times (Schmidt & Stämpfli, 1966); a similar prolongation is produced by addition of Ni^{2+} to the medium. Action potentials of up to 100 msec duration have been observed by a combination of cooling (4° C) and addition of Ni^{2+} (Meves, 1963).

SUMMARY

1. The action of scorpion venom ($10^{-8} - 10^{-6}$ g/ml.) on resting and action potentials of single myelinated frog nerve fibres was investigated.

2. In concentrations of $\geqslant 10^{-7}$ g/ml. scorpion venom produced progressive, slow and irregular depolarizations accompanied by spontaneous activity.

3. The duration of the action potential was prolonged from about 1 msec to more than 1 sec; action potentials of more than 10 sec duration have occasionally been observed.

4. Depolarization and spontaneous activity of the node were completely abolished on substitution of external sodium by choline ions, whereas replacement of sodium by lithium did not alter the response of the membrane to the venom.

5. The relation between change of resting potential and log sodium concentration of the medium (choline chloride substituting sodium chloride) had a maximum slope of 40 mV per tenfold concentration change.

6. Raising the Ca-concentration of Ringer solution repolarized the membrane partially, shortened the action potential considerably and stopped spontaneous firing. Similar effects could be observed during application of hyperpolarizing currents. With anodal pulses prolonged action potentials could be shortened in an all-or-none manner.

6. Preliminary voltage-clamp experiments with linear changes of membrane potential showed an N-shaped current-voltage relation in the range of 20 to 60 mV depolarization.

7. It is concluded that the main effect of the venom consists of an increase of the sodium permeability of the resting membrane and a delayed inactivation of the sodium permeability.

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