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THE EFFECT OF SCORPION VENOMS ON THE SODIUM CURRENTS OF THE SQUID GIANT AXON

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SUMMARY

1. The effect of externally applied scorpion venoms (0·1–0·5 mg/ml., species *Leiurus quinquestriatus* and *Centruroides sculpturatus*) on the Na currents of intracellularly perfused squid giant axons has been studied with the voltage-clamp method.

2. The venoms from the two species had the same effect. They reduced the size of the peak conductance but had little effect on its kinetics (time to peak, time constant of inactivation) and on its steady-state inactivation. The venoms increased markedly, however, the maintained conductance and the time constants of its turning-on and turning-off.

3. The voltage dependence of the maintained conductance was determined (*a*) by fitting a modified Hodgkin–Huxley equation to the Na currents and (*b*) by measuring the tail currents at the end of depolarizing pulses. The maintained conductance rose with increasing depolarization from a minimum at -20 mV to a maximum at 40 mV. The peak conductance, by contrast, was constant in the positive potential range.

4. The ratio maintained conductance in venom to maintained conductance in control varied between 2 and 7 (depending on the venom concentration and the time of treatment) and was not significantly dependent on membrane potential.

5. Peak current and maintained current reversed sign at the same potential and were both blocked by tetrodotoxin.

6. During a pulse to -2 mV preceded by a pre-pulse to -42 mV the Na conductance showed a rapid initial increase followed by a slower decay and a subsequent slow increase, reflecting the activation and inactivation of the peak conductance and the slow development of the maintained conductance.

7. Many of the observations are compatible with the idea that scorpion venoms increase the number of channels which go from the peak conductance state into the maintained conductance state (open $\rightleftharpoons$ closed $\rightleftharpoons$ open transition of the inactivation gate, see Chandler & Meves (1970*a*, *b*)). But the alternative hypothesis that peak conductance and maintained conductance reflect two separate populations of Na channels cannot be ruled out.

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INTRODUCTION

Scorpion venoms have attracted the attention of electrophysiologists because they strikingly modify the inactivation of the Na permeability. Koppenhöfer & Schmidt (1968*a*, *b*) studied the effects of the venom from the species *Leiurus quinquestriatus* on the ionic currents of the myelinated nerve fibre. After treatment with this venom, the transient Na inward current is replaced by a maintained Na inward current, and the steady-state inactivation curve turns upward at strong depolarizations (see also Schmitt & Schmidt, 1972). Subsequent work on squid giant axons (Narahashi, Shapiro, Deguchi, Scuka & Wang, 1972), on crayfish and *Sepia* axons (Romey, Chicheportiche, Lazdunski, Rochat, Miranda & Lissitzky, 1975) and on myelinated nerve fibres (Conti, Hille, Neumcke, Nonner & Stämpfli, 1976; Mozhaeva, Naumov, Soldatov & Grishin, 1979; Nonner, 1979) confirmed the finding of a large maintained inward current and a marked prolongation of the action potential, using venoms from the species *Buthus tamulus*, *Buthus eupeus*, *Androctonus australis Hector* and *Leiurus quinquestriatus*.

We were primarily interested in the effect of scorpion venoms on the steady-state Na inactivation curve (h_∞ *vs.* V relation). An increase of the h_∞ curve at positive potentials has also been observed in squid axons perfused with NaF (Chandler & Meves, 1970*a*, *b*) and has led to the hypothesis that h_∞ consists of two components, one component (h_1) which *de*creases with increasing depolarization in the manner described by Hodgkin & Huxley (1952) and another component (h_2) which *in*creases with increasing depolarization. The latter component is responsible for the maintained conductance $\bar{g}_{Na}m^3h_2$ whose voltage dependence is different from that of the peak conductance (see also Fig. 7 of Bezanilla & Armstrong, 1977). We started from the working hypothesis that scorpion venoms favour the transition of Na channels into the h_2 state, i.e. increase the maintained conductance $\bar{g}_{Na}m^3h_2$.

The experiments were done with crude dried venoms from the species *Leiurus quinquestriatus* and *Centruroides sculpturatus*, kindly supplied by Dr F. E. Russell, Los Angeles, California. Crude *Leiurus quinquestriatus* venom from Sigma (No. V-5251) was also used. The venoms from both species of scorpion had the same effect and, therefore, the results are described together.

Some of the results have been reported in a preliminary communication (Gillespie & Meves, 1978).

METHODS

Giant axons with diameters between 600 and 1000 μm were dissected from mantles of *Loligo forbesi*. The axons were perfused according to the method of Baker, Hodgkin & Shaw (1962). If excitable after perfusion, the axon was mounted horizontally in a Perspex chamber. The chamber was 20 mm long, 4 mm wide and 6 mm deep and contained 0·3–0·4 ml. artificial sea water. At both ends of the chamber the axon was suspended in air.

The voltage-clamp system was of conventional design. The internal electrode consisted of a 100 μm glass capillary for measuring the internal potential and a 75 μm platinum iridium wire for passing current. The glass capillary contained 0·6 M-KCl and a platinum wire to reduce impedance. The current wire was attached to the glass capillary and insulated except for the distal 12 mm which was carefully platinized. The external voltage electrode was a Ag-AgCl wire connected to the bath by a sea-water bridge. The external current electrode consisted of a pair of platinized platinum plates (each 2 mm in width). Two further pairs of platinized platinum plates served as guards. The feed-back amplifier was similar to that described by Bezanilla,

Rojas & Taylor (1970). The voltage-clamp currents were recorded on-line with a PDP 11 computer (Digital Equipment Corporation) and stored on cartridge disks (see Kimura & Meves, 1979). The potential was held at the holding potential for at least 3 min before records were taken. We used compensated feed-back and sea water with reduced Na concentration or with a moderate concentration of tetrodotoxin in order to reduce the error caused by the resistance in series with the membrane. Potential measurements were corrected for junction potential which was determined before and after each experiment.

The artificial sea water contained 470 mM-NaCl, 11 mM-$CaCl_2$, 55 mM-$MgCl_2$ and 5 mM-Tris-HCl buffer (pH 7·5). To reduce the Na concentration it was mixed with Na-free artificial sea water containing 524 mM-Tris (Trizma base from Sigma), 11 mM-$CaCl_2$ and 55 mM-$MgCl_2$ (pH adjusted to 7·5 with HCl). The internal solution was 218 mM-KF + 54 mM-tetraethylammonium chloride + sucrose; occasionally, the KF was replaced by a mixture of KF and NaF. The internal solutions were obtained by mixing isotonic solutions (0·6 M) of KF, NaF and tetraethylammonium chloride with an isotonic solution of sucrose (1 molal).

The temperature of the artificial sea water in the Perspex chamber was held constant at a value between 9 and 19° C and was measured with a small thermistor.

RESULTS

General description

Figs. 1 and 2 illustrate the effect of scorpion venom on two different fibres. Fig. 1 is an example of a very strong effect, Fig. 2 shows a milder effect; in Fig. 2, but not in Fig. 1, the tetrodotoxin-insensitive current has been subtracted from the total current. The venom was applied by exchanging part of the sea water in the bath with sea water with venom. Usually, a few minutes after application of the venom the fibre began to fire spontaneous action potentials whose duration increased, reaching values of 6 and 0·7 sec in the experiments of Figs. 1 and 2 respectively.

The peak Na currents in sea water with venom were smaller than in the control records; part of the decrease in Fig. 1*B* is, however, due to the absence of a hyperpolarizing prepulse. The Na currents in sea water with venom are characterized by a large maintained current. In Fig. 1*B*, the Na inward current at the pulse end is *larger* at 1 mV than at −19 mV while the peak current is *smaller* at 1 mV than at −19 mV; the current traces labelled −19 mV and 1 mV cross at 3·3 msec. The same crossing over is seen in Fig. 2*B*. Closer inspection of Fig. 2*A* suggests that the phenomenon is also present, although less pronounced, in the untreated axon.

The tail currents at the end of the depolarizing pulses are larger in Fig. 1*B* (where the smallest tail current belongs to the trace labelled −19 mV) than in the control records in Fig. 1*A*. From records on faster time base the half-time for the decay of the tail currents in Fig. 1*B* was estimated as 0·2 msec. The time to peak was not affected by the venom; e.g. the peak occurred at 0·7 msec in record labelled −24 mV of Fig. 1*A* and in record labelled −19 mV of Fig. 1*B*.

The strength of the venom effect varied considerably from fibre to fibre (as illustrated by Figs. 1 and 2), even when the same concentration of the same venom sample was used. We were not able to correlate the strength of the venom effect with other parameters (such as resting potential, size of Na currents, fibre diameter, temperature, external Na concentration) and therefore attribute it to variability between fibres. A very pronounced venom effect usually led to an early collapse of the fibre; soon after taking the records of Fig. 1*B* it was no longer possible to hold the potential and the experiment had to be ended before records in sea water with tetrodotoxin

(TTX) could be taken. Since the quantitative analysis required the subtraction of the TTX-insensitive current (leakage current, remaining K outward current), we generally aimed for a milder venom effect such as in Fig. 2. In practice, we started taking records as soon as a clear prolongation of the action potential or a clear increase of the maintained Na inward current was visible. Each experiment was limited to three or four clamp runs which were repeated immediately afterwards in the presence of 0·5 μM-TTX.

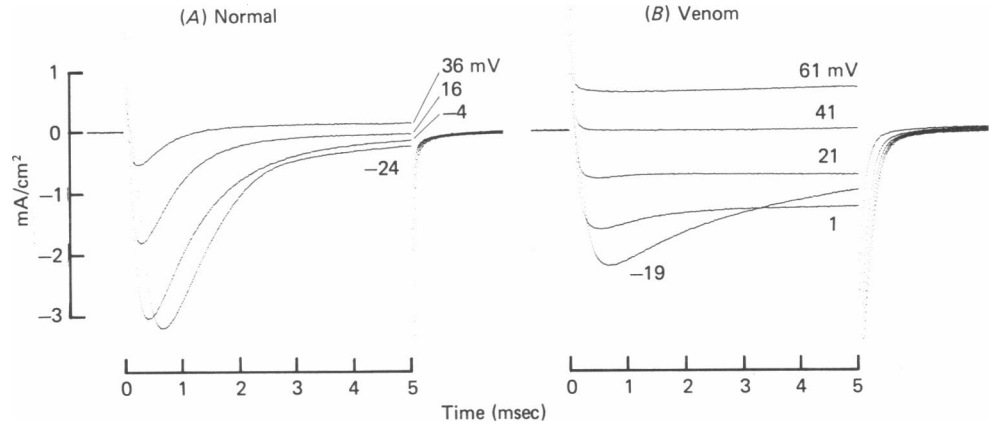

Fig. 1. Strong effect of venom (0·075 mg/ml. *Leiurus quinquestriatus*) on the Na currents. *A*, control records before application of venom. *B*, 13 min after application of sea water with venom. Resting potential −54 mV in *A*, −50 mV in *B*. Holding potential −59 mV in *A* and *B*. Potential during clamp pulses as shown. Pulses preceded by 50 msec pre-pulses to −84 mV in *A*, no pre-pulse in *B*. Full Na sea water. Temperature 15·5 °C.

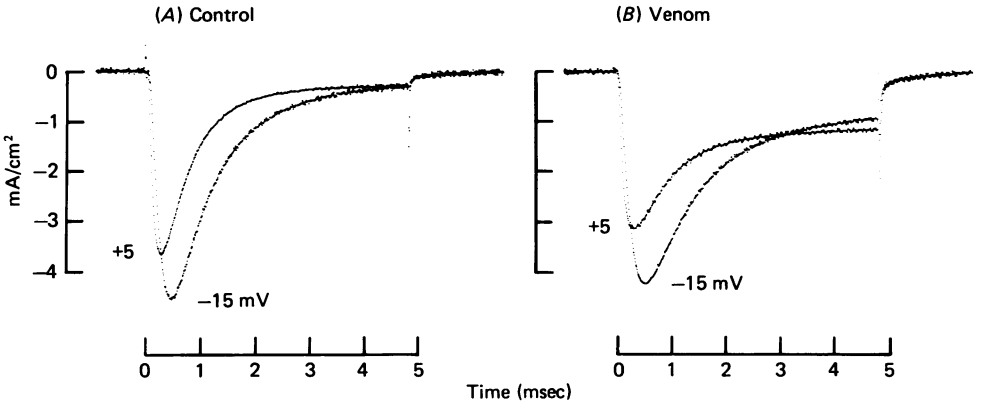

Fig. 2. Milder effect of venom (0·06 mg/ml. *Leiurus quinquestriatus*, same sample as in Fig. 1) on the Na currents. *A*, control records. *B*, 15 min after application of sea water with venom. TTX-insensitive currents, measured after the records in *B*, have been subtracted. Resting potential −55 mV in *A*, −56 mV in *B*. Holding potential −60 mV in *A* and *B*. 50 msec pre-pulse to −85 mV in *A* and *B*. Potential during clamp pulse −15 and 5 mV. Full Na sea water. Temperature 15·5 °C.

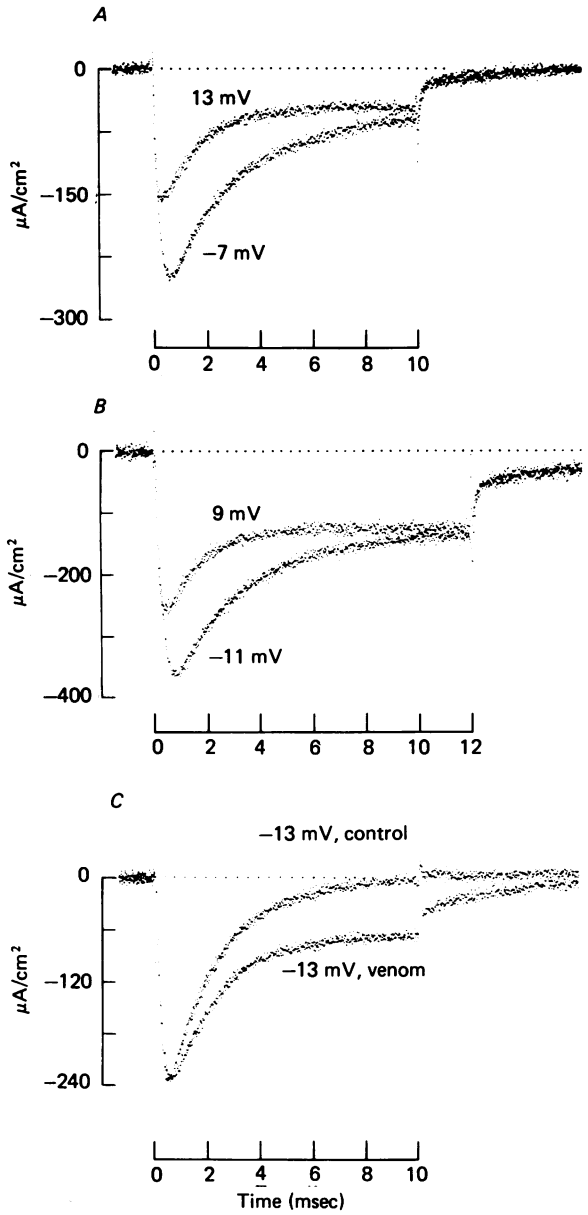

Fig. 3. Effect of venom on the Na currents in sea water with reduced Na concentration. TTX-insensitive currents subtracted. *A*, *B*, *C* from three different axons. *A*, $\frac{1}{3}$ Na sea water; currents associated with 10 msec pulses to −7 and 13 mV, recorded 10 min after application of sea water with 0·2 mg/ml. *Centruroides sculpturatus* venom; resting potential = holding potential = −67 mV; temperature 10·5 °C. *B*, $\frac{1}{3}$ Na sea water; currents associated with 12 msec pulses to −11 and 9 mV, recorded 25 min after application of sea water with 0·2 mg/ml. *Leiurus quinquestriatus* venom; resting potential = holding potential = −71 mV; temperature 11 °C. *C*, $\frac{1}{5}$ Na sea water; records are averages from five sweeps and show current associated with 10 msec pulse to −13 mV in control and 30 min after application of sea water with 0·5 mg/ml. *Leiurus quinquestriatus* venom (from Sigma); resting potential −73 mV in control, −76 mV in venom; holding potential −73 mV in both; temperature 19 °C. Fitting the currents in *C* with eqn. (1) gave

	h_∞	τ_h
for control	0·002	1·97 msec
for venom	0·204	1·94 msec

Peak current and maintained current

The different voltage dependence of the (large) peak current and the (smaller) maintained current shown in Fig. 2 *B* could be due to an error caused by the uncompensated part of the series resistance (see effect of series resistance on current voltage curves in Figs. 26 and 27 of Taylor, Moore & Cole, 1960). The voltage drop across the uncompensated part of the series resistance will also distort the tail currents at the end of the clamp pulses (see Fig. 3 of Ramón, Anderson, Joyner & Moore, 1975). We tried to minimize these errors by using $\frac{1}{3}$ or $\frac{1}{5}$ Na sea water (in addition to compen-

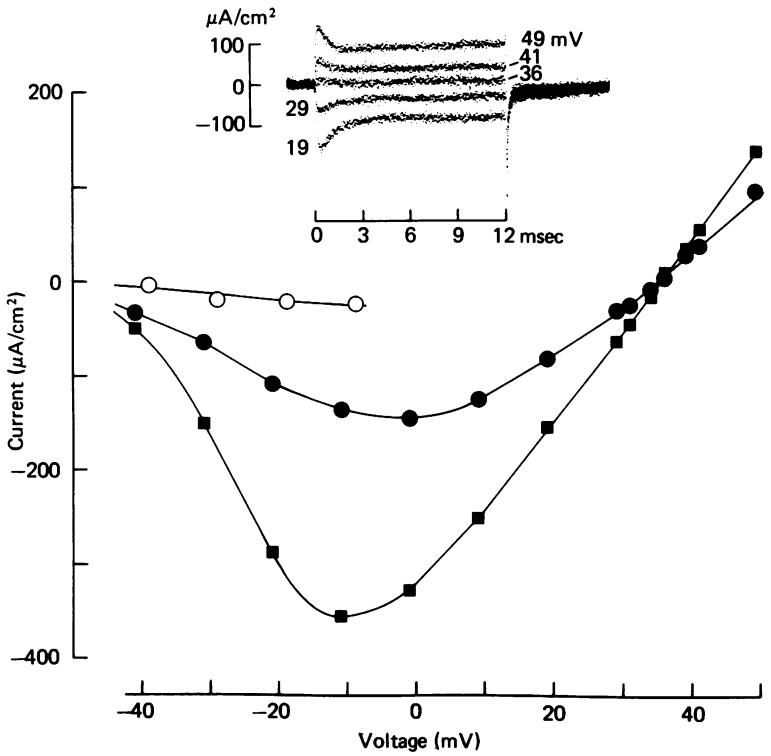

Fig. 4. Current–voltage curves for the peak current (■) and the maintained current (●, measured at 12 msec) in a fibre treated with venom. Same experiment as Fig. 3 *B*. TTX-insensitive currents subtracted. Curves through ■ and ● drawn by eye. Inset shows original records from the same experiment with pulse potentials below and above the reversal potential. The graph shows also a few values (○) for the maintained current before application of venom; curve through ○ obtained by dividing curve through ● by 6.

sated feed-back). Fig. 3 *A* and *B* shows currents (minus TTX-insensitive currents) from two venom-treated fibres in $\frac{1}{3}$ Na sea water. Increasing the pulse height by 20 mV leads to a clear decrease of the peak current (due to reduction of the driving force for Na ions) whereas the current at the end of the 10 or 12 msec pulses is little changed; in Fig. 3 *B*, extrapolation of the two traces to longer times suggests in fact an *in*crease of the maintained current. We conclude that the different voltage dependence of peak current and maintained current is not an artifact caused by the series resistance.

Likewise, the effect of venom on the tail currents can be reproduced in low Na sea water. Slow tail currents after venom treatment are visible in Fig. 3*A* and especially in Fig. 3*B*. Fig. 3*C* shows the current during a pulse to −13 mV and following repolarization to −73 mV before and after venom treatment in $\frac{1}{5}$ Na sea water. Venom slightly reduced the peak current, but markedly increased the maintained

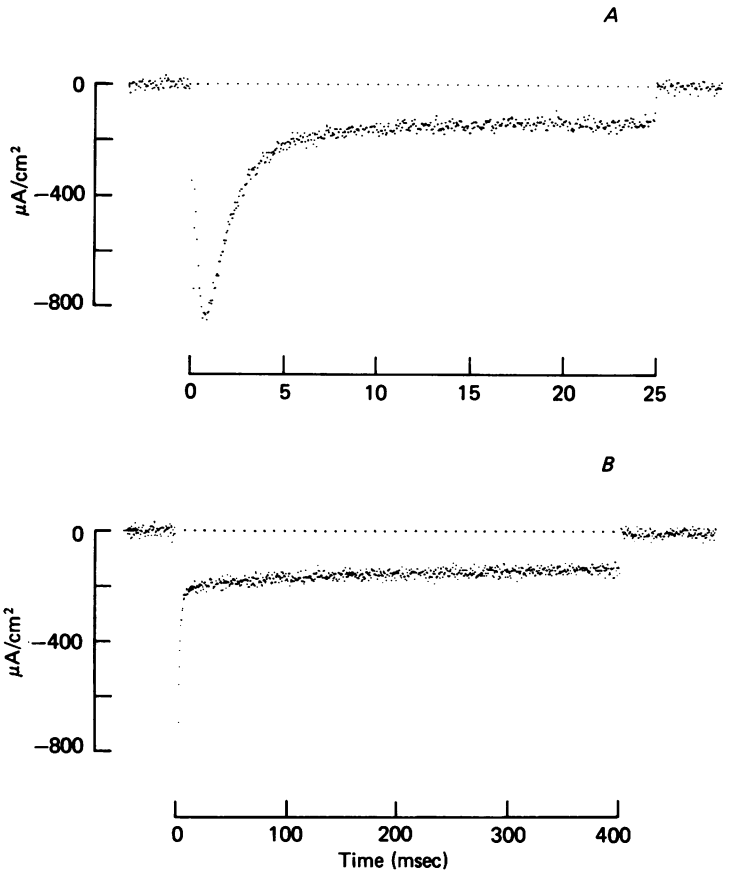

Fig. 5. Na current associated with a 25 msec (*A*) and a 400 msec (*B*) pulse to −20 mV in a venom-treated axon. Recorded 15 min after application of sea water with 0·05 mg/ml. *Leiurus quinquestriatus* venom. TTX-insensitive current not subtracted. Resting potential −49 mV, holding potential −70 mV; 50 msec pre-pulse to −90 mV. Full Na sea water. Temperature 16 °C. Record *A* taken after record *B*.

current and produced a large tail current which decreased very slowly (half-time 1·9 msec) in spite of the high temperature (19 °C).

It is noteworthy that the effect shown in Fig. 3*C* was obtained using a relatively high concentration of venom from Sigma. In all other Figures venoms from Dr Russell were used, and these acted at much lower concentrations.

The peak current (■) and the maintained current (●) measured after treatment with venom in the experiment of Fig. 3*B* are plotted against pulse potential in Fig. 4. The maximum of the peak current and of the maintained current occur at

−11 and −1 mV, respectively. The peak current is smaller at 9 mV than at −21 mV while the maintained current is larger. The reversal potentials for peak current and maintained current are, however, identical within half a millivolt (35·3 mV for the peak current, 34·8 mV for the maintained current). Equality of the reversal potentials is also illustrated by the original records in the inset of Fig. 4. Consequently, the different voltage dependence of peak current and maintained current must reflect a different voltage dependence of the underlying conductances. Information about the voltage dependence of the maintained conductance will be given in the following sections.

We tried to evaluate the venom effect by comparing the maintained current (minus TTX-insensitive current) before and after application of venom. Four control values for the maintained current (○) are given in Fig. 4; dividing the curve through ● by 6 gave an approximate fit of the control values (○). For stronger depolarizing pulses accurate control values for the maintained current were difficult to obtain. Their measurement required subtraction of relatively large TTX-insensitive currents (leakage current, remaining K outward current) and the latter were recorded *in sea water with venom*, a procedure which must lead to an error if venom affects the TTX-insensitive current. A large difference between the reversal potentials for peak current and maintained current in the control records suggested to us that such an error did indeed occur, and we therefore give only the control values for small depolarizing pulses. The ratio maintained current in venom to maintained current in control (which was 6 in Fig. 4) varied in different experiments according to the strength of the venom effect. In no case did it seem to depend on membrane potential.

The decay of the Na currents in Fig. 3*A–C* could be described by a single time constant. This was true for most experiments with 10–12 msec pulses. Only occasionally were currents observed which required two time constants for a proper fit of the falling phase (e.g. record + 5 mV in Fig. 2*B*). However, records with 400 msec pulses (Fig. 5*B*) showed that the relatively rapid initial decay is followed by a very slow decline of the 'maintained' current similar to the slow decline described by Chandler & Meves (1970*c*) for NaF-perfused axons. The slow decline was also seen in the control record (not shown), but the 'maintained' current was three times smaller and decayed to zero within about 400 msec. The current associated with a 25 msec pulse (Fig. 5*A*) appeared to follow an exponential time course, but a semilogarithmic plot revealed a slight deviation from a straight line, probably because of the slow decline of the 'maintained' current.

The effect of scorpion venom (0·1 mg/ml. *Leiurus quinquestriatus* venom for 20 min) on the maintained current was partially reversible during a washout period of 17 min. Internal application in concentrations of 0·13–0·26 mg/ml. had no effect; this confirms the results of Narahashi *et al.* (1972).

h_∞ and τ_h from curve fit

The currents (minus TTX-insensitive currents) associated with 10–12 msec pulses were fitted with the equation (see Kimura & Meves, 1979):

$$I_{Na} = I'_{Na}[1-\exp(-t/\tau_m)]^3[h_\infty(1-\exp(-t/\tau_h))+\exp(-t/\tau_h)], \quad (1)$$

where

$$I'_{Na} = P'_{Na} \frac{F^2V}{RT} [Na]_0 \frac{\exp((V - V_e)F/RT) - 1}{\exp(VF/RT)} \quad (2)$$

and

$$P'_{Na} = \bar{P}_{Na} m^3_\infty.$$

This equation applies to the case where $m_0 = 0$ and $h_0 = 1$. No account was taken of the very slow decline of the 'maintained' current. The four adjustable parameters (I'_{Na}, τ_m, h_∞, τ_h) were determined by the computer. Goodness of fit was evaluated from the standard deviations of the curve-fit parameters. When the standard deviation was greater than 10 % the value was rejected. In general, the curve fits showed a decrease of I'_{Na} in venom, no significant effect on τ_m, a large but variable increase of h_∞ and a mild increase of τ_h (Table 1).

TABLE 1. Effect of scorpion venoms on I'_{Na}, τ_m, h_∞ and τ_h at a potential between 0 and 6 mV, determined by fitting the currents (after subtraction of the TTX-insensitive current) with eqn. (1). Values measured in the absence and in the presence of venom are indicated by the subscript contr and ven, respectively. All experiments with $\frac{1}{3}$ Na sea water at 9–11 °C and with *Centruroides sculpturatus* venom (except experiments *e* and *f* which were done with *Leiurus quinquestriatus* venom). Pulse length 10–12 msec, except in experiment *d* where 30 msec pulses were used. Records from experiments *a* and *e* are shown in Fig. 3*A* and *B*, respectively

Expt.	V (mV)	$\frac{I'_{Na,ven}}{I'_{Na,contr}}$	$\frac{\tau_{m,ven}}{\tau_{m,contr}}$	$h_{\infty,contr}$	$h_{\infty,ven}$	$\frac{h_{\infty,ven}}{h_{\infty,contr}}$	$\tau_{h,contr}$ (msec)	$\tau_{h,ven}$ (msec)	$\frac{\tau_{h,ven}}{\tau_{h,contr}}$
a	3	0·76	0·94	0·06	0·20	3·17	1·72	1·87	1·09
b	6	0·74	0·96	0·08	0·35	4·59	2·33	2·58	1·11
c	0	0·87	1·06	0·08	0·15	1·84	2·23	2·78	1·25
d	5	0·68	1·66	0·12	0·32	2·70	1·59	2·01	1·27
e	1	0·88	1·13	0·05	0·33	6·96	1·44	1·83	1·27
f	6	1·05	1·12	0·08	0·24	3·15	1·69	2·02	1·20

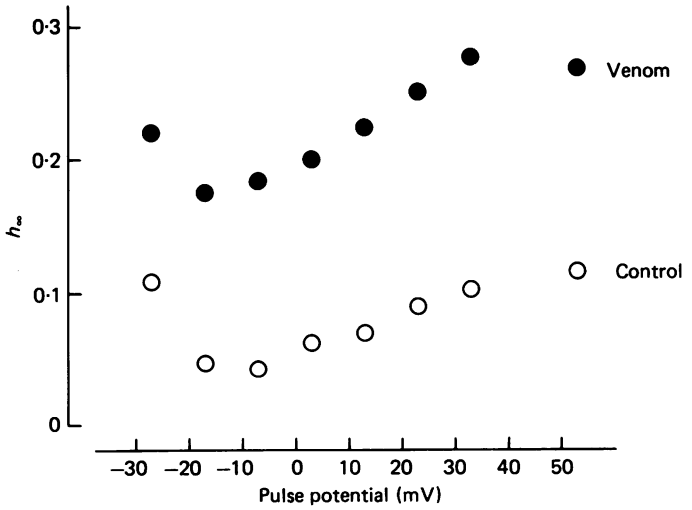

Fig. 6. Inactivation variable h_∞ (obtained by curve fit) as a function of pulse potential for control (○) and venom (●). Same experiment as in Fig. 3*A*. Values near reversal potential (= 38 mV) not plotted.

The h_∞ values (○ control, ● venom) found in the experiment of Fig. 3*A* ($\frac{1}{3}$ Na sea water, 10·5 °C) are plotted in Fig. 6 against pulse potential. The control values (○) show a minimum of h_∞ at small negative potentials (cf. Fig. 11 of Chandler & Meves, 1970*b*); again, the subtraction of relatively large TTX-insensitive currents causes some uncertainty of the control values. Venom (●) substantially increased h_∞ without altering its voltage dependence. At 3 mV h_∞ was increased by a factor of 3·17. The time constant τ_h measured at 3 mV increased only by a factor of 1·09. Table 1 (which contains the experiment of Figs. 3*A* and 6 as experiment *a*) shows that this finding was typical: h_∞ (measured at a potential between 0 and

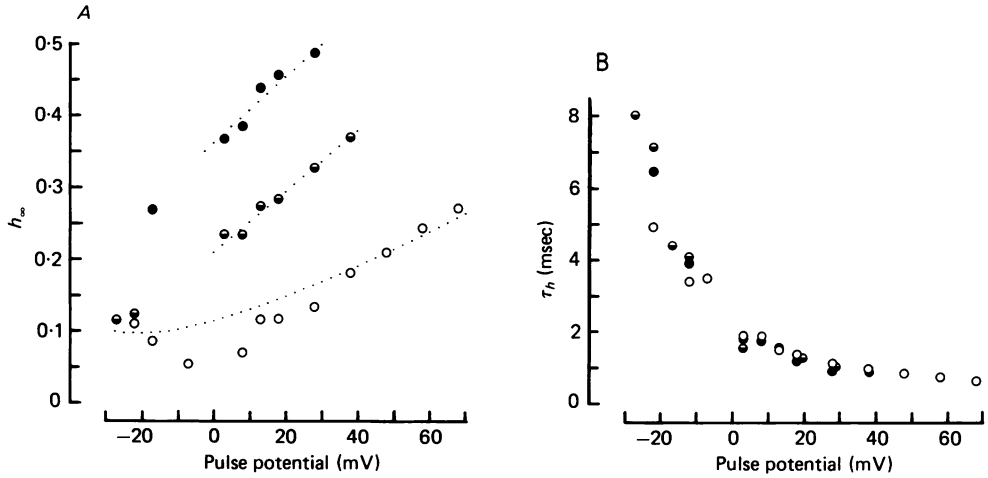

Fig. 7. Inactivation variable h_∞ (in *A*) and time constant τ_h (in *B*) (obtained by curve fit) as a function of pulse potential. ○, control. ◒ and ●, 13 and 22 min after external application of 0·24 mg/ml. *Leiurus quinquestriatus* venom. Internal solution: 146 mM-NaF + 72 mM-KF + 54 mM-tetraethylammonium chloride + sucrose. Resting potential − 52 mV in control, − 49 mV in venom. Holding potential − 62 mV and 88 msec pre-pulse to − 67 mV in all three runs. Pulse duration 12 msec. $\frac{1}{3}$ Na sea water. Temperature 10 °C. The dotted curve through ○ was obtained by multiplying the $(h_{1\infty} + h_{2\infty})$ curve in Fig. 11 of Chandler & Meves (1970*b*) by 0·75. The dotted curves through ◒ and ● were drawn by eye.

6 mV) increased by a factor between 2 and 7 while the time constant τ_h increased only 1·1–1·3 times (see also time constants in legend of Fig. 3*C* which indicate a change of τ_h by a factor of 0·98).

In the six experiments of Table 1 the time constant τ_h was also determined by varying the duration of a conditioning pre-pulse. The pre-pulse potential was between − 50 and 21 mV. Small conditioning pre-pulses preceded the test pulse directly, large pre-pulses were separated from the test pulse by a 4–5 msec gap (cf. Gillespie & Meves, 1980). The average ratio $\tau_{h\,\text{venom}}/\tau_{h\,\text{control}}$ was 1·13, suggesting again a small increase; the extreme values of the ratio were 0·85 and 1·48. Even in the experiment of Fig. 1, which shows a very strong venom effect, the time constant τ_h (measured with conditioning pre-pulses to − 39 mV) was only increased by a factor of 1·02.

Fig. 7 shows h_∞ and τ_h, again obtained by curve fit, for a fibre perfused with a mixture of NaF and KF. The reversal potential was now − 1 mV as opposed to

38 mV in Fig. 6, i.e. the gap in the positive potential range which is seen in Fig. 6 was avoided. The control values (○) for h_∞ in Fig. 7*A* are approximately fitted by the dotted curve; it was calculated by multiplying the $(h_{1\infty}+h_{2\infty})$ curve in Fig. 11 of Chandler & Meves (1970*b*) (which is for a NaF-perfused axon) by 0·75. The factor 0·75 accounts for the fact that α_{h_2}/β_{h_2} (and therefore $h_{2\infty}$) is smaller with internal KF than with internal NaF (Chandler & Meves, 1970*b*). (A factor of about 0·4 would be needed to fit the points ○ in Fig. 6 which were measured in a KF-perfused axon.) Venom substantially increased h_∞ (Fig. 7*A*); the effect was more pronounced after 22 min application (●) than after 13 min (◒). Again, there was little effect on the time constant τ_h (Fig. 7*B*) and no effect on τ_m (not shown). $P'_{Na} = \bar{P}_{Na} m^3{}_\infty$, calculated from I'_{Na} and the reversal potential V_e by means of eqn. (2), increased steeply in the range of negative potentials. At positive potentials, P'_{Na} was constant. This was true for the measurements after 13 min and for those after 22 min venom application and contrasts with the marked increase of h_∞ for $V > 0$ mV, demonstrating again the different voltage dependence of peak conductance and maintained conductance described in the preceding section. P'_{Na} at 18 mV was 6·75, 5·25 and $4{\cdot}57 \times 10^{-5}$ cm/sec in control, after 13 min and after 22 min venom application respectively, suggesting a 22–32 % decrease of $\bar{P}_{Na}$ in venom.

Double pulses without gap

The increase of the maintained current between −20 and 10 mV as shown in Figs. 1*B* and 2*B* can be demonstrated by a double-pulse experiment as in Fig. 8 (which is from the same axon as Fig. 2). Traces *b* in Fig. 8*A* (control) and *B* (venom) were obtained with a 20 msec pre-pulse to −20 mV followed by a 4·7 msec pulse to 10 mV. Trace *b* of *B* (venom) shows the maintained inward current during the last 1·5 msec of the pre-pulse to −20 mV and its slow increase during the further depolarization to 10 mV. (Subtraction of the TTX-insensitive current from trace *b* of *B* (venom) removed most of the brief upward transient at the beginning of the pulse to 10 mV.) For comparison, the current associated with a single pulse from the holding potential (−60 mV) to 10 mV is presented as trace *a*. At the end of the 4·7 msec pulse to 10 mV traces *a* and *b* have almost reached the same level, but from opposite sides. An increase of the maintained inward current during a depolarization from −20 to 10 mV is also just visible in trace *b* of *A* (control). It seems that venom merely enhances a phenomenon which is already present in the untreated axon.

When the pulse potential 10 mV was approached from a more positive potential value such as 40 mV (Fig. 8*C*, trace *c*), a slowly decaying tail of inward current was observed, representing the decrease of the maintained conductance from its high value at 40 mV to its lower value at 10 mV. Eventually, the current reached the same level as the current associated with a step from −20 to 10 mV (Fig. 8*C*, trace *b*). Traces *b* and *c* were fitted by two exponentials (time constants 0·13 and 1·31 msec) and one exponential (time constant 1·45 msec), respectively.

The experiment of Fig. 8, thus, demonstrates slow current changes, presumably due to slow changes of the maintained conductance, in the potential range −20 to 40 mV. Fig. 9 shows another double-pulse experiment in which pre-pulses to more negative and to more positive potentials than in Fig. 8 were used. The records were taken in sea water with venom and with a moderate TTX concentration (which

served to reduce the size of the Na currents and thereby the error produced by the series resistance); the TTX-insensitive current, measured after application of a high TTX concentration at the end of the experiment, was subtracted. The records show the current during the last 1·9 msec of the pre-pulse (going to different potentials) and during a subsequent pulse to −2 mV. Trace *b* of Fig. 9 (pre-pulse potential −32 mV) illustrates the increase of the maintained inward current during a de-

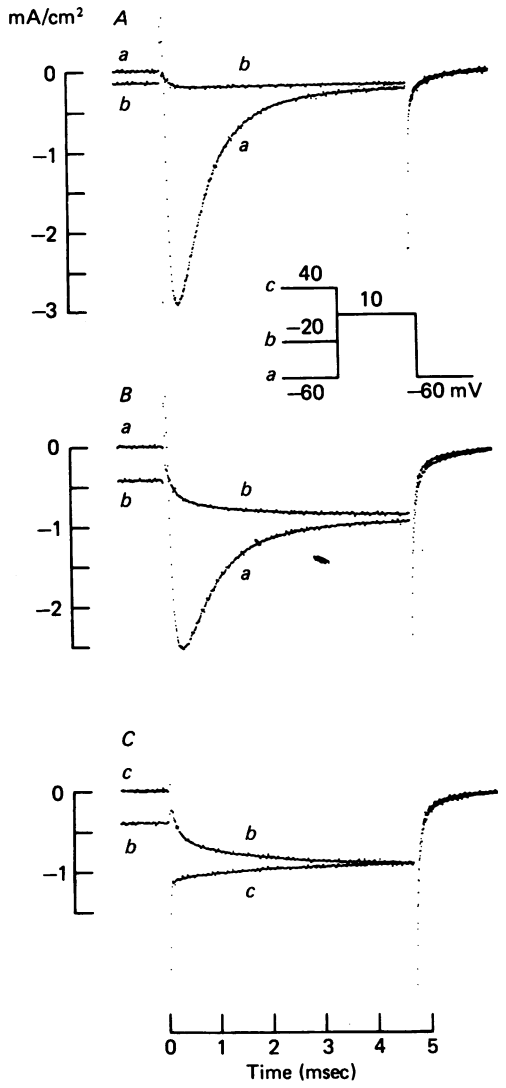

Fig. 8. Double-pulse experiment with pulse programme as shown in inset: *a*, single pulse from holding potential −60 mV to 10 mV; *b* and *c*, pulse to 10 mV preceded by 20 msec pre-pulse to −20 or 40 mV. Records show current during last 1·5 msec of pre-pulse and during pulse to 10 mV. *A*, control, traces *a* (no pre-pulse) and *b* (pre-pulse to −20 mV). *B*, 15 min after application of sea water with 0·06 mg/ml. *Leiurus quinquestriatus* venom, traces *a* (no pre-pulse) and *b* (pre-pulse to −20 mV). *C*, again 15 min after application of venom, traces *b* (pre-pulse to −20 mV) and *c* (pre-pulse to 40 mV). Same experiment as Fig. 2.

polarization from −32 to −2 mV. As in trace *b* of Fig. 8 *B* and *C* the increase occurred in two phases, an initial jump (possibly indicating an increase of *m*) and a much slower second phase. Following a pre-pulse to 78 mV (Fig. 9, trace *c*), there was a large tail of inward current, decaying slowly to a level close to that reached by trace *b*. The time constants of the slow current changes in traces *b* and *c* were estimated from the half times as 10·82 and 4·67 msec, respectively. With pre-pulse potentials between −22 and 58 mV the estimated time constants for the slow current change ranged between 6·5 and 8·5 msec and did not show a significant dependence on pre-pulse potential. The relatively small time constant estimated for trace *c* may be due to the use of a very strong pre-pulse.

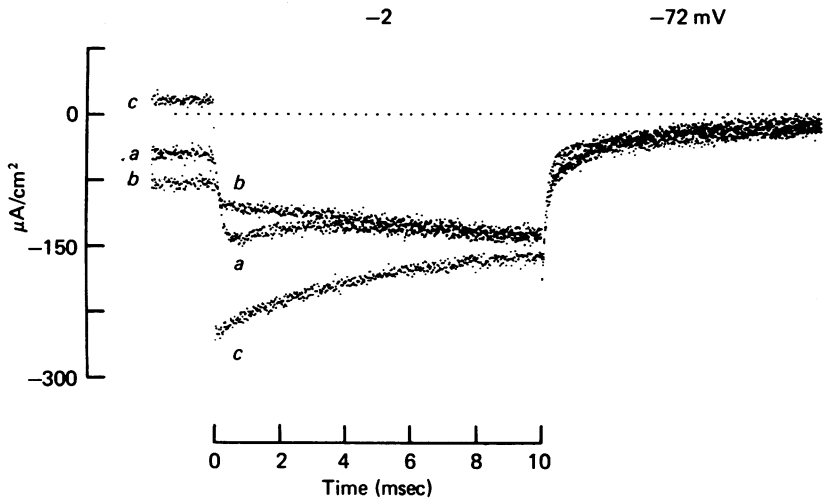

Fig. 9. Another double-pulse experiment. Records taken 12 min after application of sea water with 0·2 mg/ml. *Leiurus quinquestriatus* venom. TTX-insensitive currents subtracted. Resting potential = holding potential = −72 mV. Pulse programme: 30 msec pre-pulse to different potentials (*a*, −42 mV; *b*, −32 mV; *c*, 78 mV), followed by a 10 msec pulse to −2 mV. Records show current during last 1·9 msec of pre-pulse and during pulse to −2 mV. Full Na sea water with a moderate TTX concentration which reduced peak Na current to about a fifth of its normal height. Temperature 11 °C.

In trace *a* of Fig. 9 the pre-pulse was only to −42 mV. The test pulse to −2 mV produced a fast rising small peak of inward current (at 0·7 msec), followed by a slow decay which (at about 4 msec) became a slow increase. Trace *a* is best explained by assuming two different conductances: a peak conductance which inactivates and a maintained conductance which develops more slowly and does not appear to inactivate.

A complete separation of peak and maintained conductance was not possible. In order to separate the two conductances as far as possible, the following method was used. We measured the peak Na inward current (starting from the zero current line) in those records which showed at least a small peak of inward current (e.g. trace *a* in Fig. 9) and plotted peak I_{Na} as a function of pre-pulse potential (Fig. 10*A* with ○ for control and ● for venom); this covered the range of pre-pulse potentials more negative than −20 mV. When there was no peak inward current (e.g. traces *b* and *c* in Fig. 9), we extrapolated the slow tail current flowing during the test pulse (pulse

potential −2 mV) to the beginning of the test pulse and plotted I_0 as a function of pre-pulse potential (Fig. 10*B*); this was done for positive and small negative pre-pulse potentials.

Fig. 10 shows clearly that venom had little effect on peak I_{Na} but increased the tail current I_0 (which is a measure of the maintained conductance at the end of the pre-pulse) drastically. Dividing the points in Fig. 10*A* by the peak current measured at a pre-pulse potential of −102 mV gives the steady-state inactivation curve h_∞ *vs.* *V*. The potential for half-inactivation (h_∞ = 0·5) is −51·5 and −48·5 mV for the

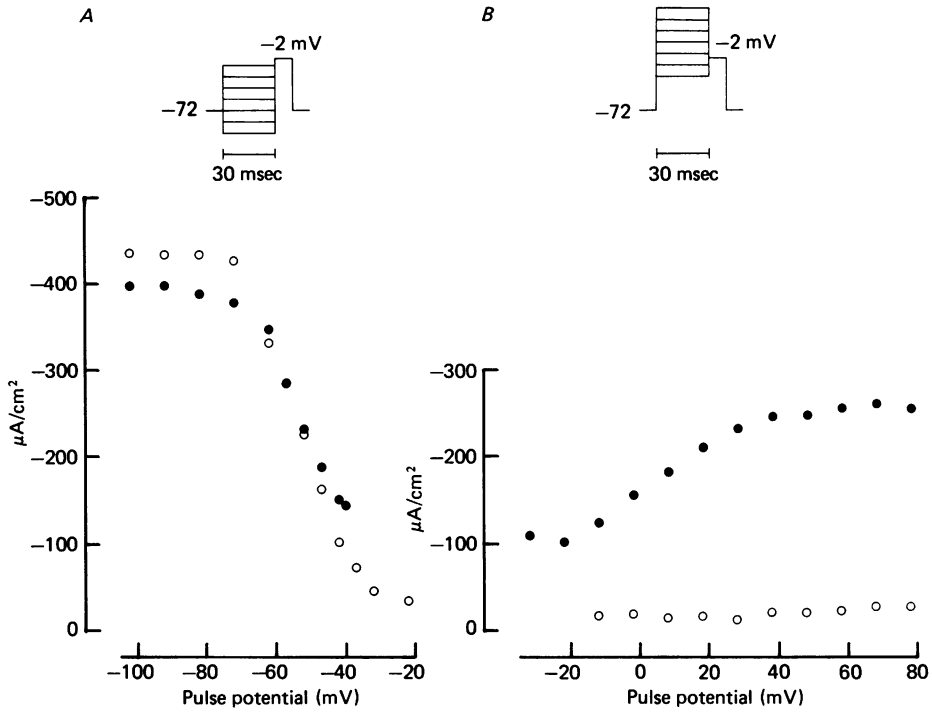

Fig. 10. Analysis of the double-pulse experiment of Fig. 9. ○, control; ●, venom. *A*, peak Na inward current as a function of pre-pulse potential; pre-pulse potential more negative than test pulse potential (see pulse programme in inset). *B*, tail current (extrapolated to beginning of test pulse) as a function of pre-pulse potential; pre-pulse potential similar to or more positive than test-pulse potential (see pulse programme in inset).

points ○ and ●, respectively. A clear effect of venom is only seen at −40 mV, where h_∞ is 0·20 for control and 0·36 for venom. At this potential the maintained current in venom begins to appear (see Fig. 4) and the peak Na inward current (measured from the zero-current line) may thus be over-estimated. Results similar to those in Fig. 10*A*, suggesting little effect of venom on the steady-state inactivation of the peak current, were obtained in two other experiments.

The large increase of the tail current I_0 (shown in Fig. 10*B*) confirms Fig. 1 of the preliminary communication (Gillespie & Meves, 1978). As in the latter Figure, the magnitude of I_0 in venom (●) seems to saturate at strong positive pre-pulse potentials; there may be a slight further increase at even stronger potentials and we shall

therefore call the phenomenon apparent saturation. The tail currents I_0 in control (○) are smaller in Fig. 10*B* than in Fig. 1 of Gillespie & Meves (1978) (because the Na currents in the experiment of Fig. 10 were reduced by TTX) and show only a small increase with increasing pre-pulse potential; on closer inspection, the values at 68 and 78 mV are 1·6 times larger than the average of the four values between −12 and 18 mV.

That venom markedly increases the size and time constant of the tail current is also evident from comparing record 'venom' in Fig. 11*A* (identical with trace *c* in Fig. 9) with record 'control'. Fig. 11*B* serves to test whether I_0 in venom really

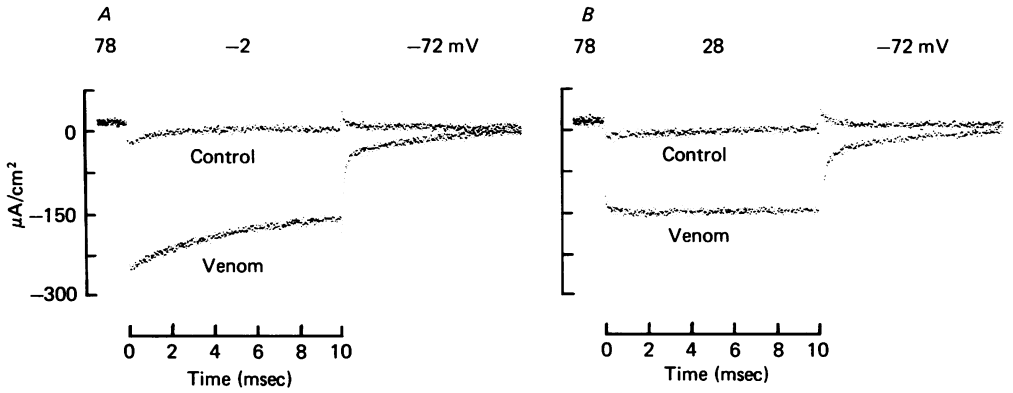

Fig. 11. Test for saturation of the venom-induced maintained conductance at strong positive potentials. Continuation of the experiment of Figs. 9 and 10. Pulse programme: 30 msec pre-pulse to 78 mV, followed by a 10 msec pulse to −2 mV (*A*) or 28 mV (*B*). TTX-insensitive current subtracted. Records show current (in control and in venom) during last 1·4 msec of pre-pulse and during pulse to −2 or 28 mV. Note slow decay of inward current labelled 'venom' in *A* and horizontal time course in *B*.

saturates at $V > 30$ mV as seen in Fig. 10*B*. The pulse programme in Fig. 11*B* is the same as in Fig. 11*A*, except that the test-pulse potential is 28 mV instead of −2 mV. Upon changing the potential from 78 to 28 mV the current 'venom' (whose reversal potential is at 70 mV) immediately changes from a small outward current to a large inward current but stays constant thereafter, indicating that the venom-induced conductance at 28 mV is very nearly the same as that at 78 mV (in confirmation of Fig. 10*B*). This is in contrast to Fig. 11*A* where the inward current during the test pulse to −2 mV decreases slowly (time constant 4·67 msec) as the conductance decays from the high value attained at 78 mV to the smaller value at −2 mV. The currents 'control', on the other hand, show a small decrease both at −2 mV (Fig. 11*A*, time constant 1·63 msec) and at 28 mV (Fig. 11*B*); in Fig. 11*B* the current 'control' is 22 μA/cm^2 at the beginning of the test pulse and 1·5 μA/cm^2 at its end. It is difficult to be sure because the currents are so small, but Fig. 1 of Gillespie & Meves (1978) (which is based on full-size Na currents) also shows no saturation of the control current tails.

Double pulses with gap

Further evidence for a conductance increase in the positive potential range and for apparent saturation of this conductance in venom-treated axons was obtained by using double pulses with a gap between the first (variable) pulse and the second (constant) pulse. As illustrated in Fig. 7 of Chandler & Meves (1970*a*) and in Fig. 8 of Bezanilla & Armstrong (1977), the size of the current produced by the second pulse (I_2) depends on the height of the first pulse: I_2 is *larger* for a first pulse to, say, 100 mV than for a first pulse to about 0 mV. Fig. 12*A* shows the above effect in an

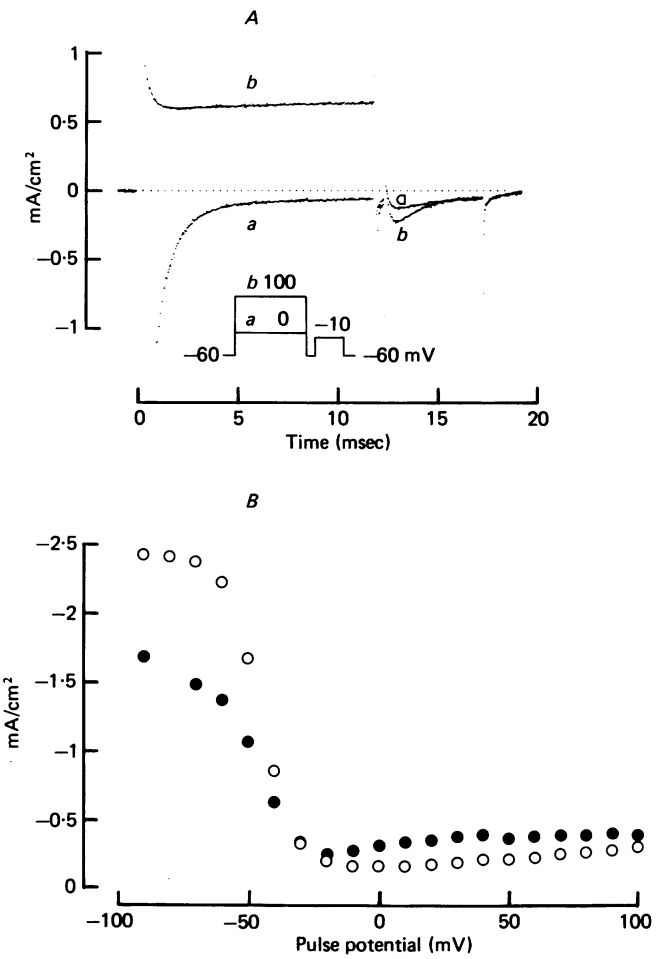

Fig. 12. Experiment using double pulses with a gap of 0·6 msec. First pulse 11·8 msec long and of varying height, second pulse 4·9 msec long and of constant height (to −10 mV). *A*, records before application of venom, first pulse to 0 mV (trace *a*) or to 100 mV (trace *b*); peaks of Na current during first pulse beyond scale; TTX-insensitive current not subtracted. *B*, current produced by second pulse (I_2) plotted against potential during first pulse; ○, control; ●, venom (12 min after addition of sea water with 0·15 mg/ml. *Centruroides sculpturatus* venom); TTX-insensitive current subtracted. *A* and *B* from same experiment. Resting potential −62 mV in control, −64 mV in venom; holding potential −60 mV in both. Full Na sea water. Temperature 16 °C.

untreated axon at 16 °C, using a gap of 0·6 msec between the two pulses; the first pulse was to 0 mV in *a* (producing an inward current) and to 100 mV in *b* (producing an outward current); the peak inward current I_2, associated with the second pulse (to −10 mV), is larger in trace *b* than in trace *a*. In Fig. 12*B*, the current I_2 (now minus TTX-insensitive current) is plotted against the potential during the first pulse for the untreated axon (○) and for the same axon after application of venom (●). The points ○ show a minimum at −10 to 10 mV and increase slightly for more positive potentials. After treatment with venom (points ●) the increase is more pronounced, but the points seem to saturate at first pulse potentials above 40 mV. In the range of negative first pulse potentials, the control values (○) increase steeply between −30 and −70 mV and reach a maximum at −100 mV while the venom values (●) grow more gradually and approach a smaller maximum; the potential for half-maximum is −44 mV for ○ and −45 mV for ●.

In another axon the gap length was increased to 2·2 msec. With this long gap the phenomenon was no longer present in the untreated axon (temperature 15 °C) but became clearly visible after treatment with venom. In the same axon, the time constant for removal of inactivation (determined at −70 mV by varying the gap between two 4·8 msec pulses to −19 mV) was 3·00 msec for control and 3·07 msec after venom treatment. This shows that the different behaviour of untreated and venom-treated axon is not due to a faster recovery from inactivation during the 2·2 msec gap.

DISCUSSION

Scorpion venoms increase the maintained conductance markedly and slow its kinetics. The peak conductance is often reduced by the venoms, indicating a reduction in the number of active Na channels, but is otherwise little affected: the time constant of Na activation (τ_m) is not changed; the time constant of inactivation of the peak conductance (τ_h) is only slightly increased (factor 1·0–1·3 from decay of peak current, factor 1·13 from double-pulse experiments); the rate of removal of inactivation is little affected; the steady-state inactivation of the peak conductance and the potential for half-inactivation are not altered significantly (Figs. 10*A* and 12*B*).

As noted on p. 481, the effectiveness of the same venom sample varied between different axons. In addition we observed that venoms from different sources had different potency (see p. 485). Such variability might be expected: the crude venoms consist of a variety of small polypeptides (Miranda, Kupeyan, Rochat, Rochat & Lissitzky, 1970; Babin, Watt, Goos & Mlejnek, 1975; Tu, 1977). Venoms from different sources may contain different proportions of the active peptide. Such an explanation may also account for the fact that the peculiar effect of *Centruroides sculpturatus* venom seen by Cahalan (1975) in nodes of Ranvier (characterized by a Na inward current developing after the termination of a depolarizing pulse and interpreted as a shift of the m_∞ curve to more negative potentials) was not seen in our experiments.

Comparison with previous work on squid axons (Narahashi *et al.* 1972) reveals agreement on some points but disagreement in others. We agree with the conclusion that the *m* system (Na activation) is little affected (see also Conti *et al.* 1976, p. 739) and with the observation that the voltage dependence of the maintained conductance is different from that of the peak conductance (see Fig. 6 of Narahashi *et al.* 1972).

The disagreement concerns mainly the reversal potential of the maintained current and the time constant of inactivation of the peak conductance (τ_h). In our experiments peak current and maintained current reverse sign at the same potential whereas Narahashi *et al.* find the reversal potential of the maintained current more positive by an average of 24 mV; we think that this difference may be an artifact due to subtraction of a linear leakage current, a possibility discussed by Narahashi *et al.* (see p. 856 of their paper). In our experiments the time constant τ_h for the decay of the peak current increased only by a factor of 1·0–1·3 whereas replotting the current traces in Fig. 8 of Narahashi *et al.* reveals an increase from 0·15 msec (control) to 0·60 msec (venom), i.e. by a factor of 4·0. The extraordinary small value of the control time constant (measured at 0 mV and 10 °C) of Narahashi *et al.* suggests to us a distortion of the falling phase of the control record by the series resistance (see Fig. 1*a* of Binstock, Adelman, Senft & Lecar, 1975). We think that those of our measurements which gave factors $> 1{\cdot}2$ may also be subject to series resistance error because an experiment in $\frac{1}{5}$ Na gave a factor close to 1·0 (see Fig. 3*C*). Reduction of the peak current by venom will reduce the voltage drop across the series resistance, thereby bringing τ_h closer to its real value, i.e. producing an apparent increase of τ_h.

The strong venom effect in Fig. 1*B* is characterized by an almost rectangular time course of the Na inward currents at 1 and 21 mV, resembling the records from myelinated nerve fibres treated with scorpion venom (Koppenhöfer & Schmidt, 1968*a*; Conti *et al.* 1976). The slow decay of the trace labelled −19 mV and the almost horizontal currents at more positive pulse potentials may result from the inactivation of the peak conductance and the simultaneous turning-on of the maintained conductance. It is noteworthy that even in the experiment of Fig. 1 with its strong venom effect the time constant τ_h (determined by measuring the peak Na currents following conditioning pre-pulses of varying length to a potential of −39 mV) was not significantly increased (see p. 488). A full quantitative analysis of the strong venom effect was not feasible since the axons did not survive long enough.

We determined the voltage dependence of the maintained conductance by two different methods: (*a*) by measuring the instantaneous value of tail currents following long pulses of different size, (*b*) by fitting the currents associated with depolarizing pulses of different size with eqn. (1). With the first method the maintained conductance was found to increase with increasing potential and to reach an apparent saturation value at about 40 mV (Fig. 10*B*). Similarly, with the second method we found a marked increase of h_∞ in the potential range −10 to 40 mV (Figs. 6 and 7). The increase of the maintained conductance at positive potentials contrasts with the constancy of the peak conductance $P'_{Na} = \bar{P}_{Na} m^3{}_\infty$ in this potential range (see p. 489

The maintained conductance turns on and off very slowly. When the peak conductance had been inactivated by a sufficiently long (20–30 msec) and strong depolarizing pre-pulse, the time constant of the maintained conductance could be estimated. The values obtained in the potential range −7 to 10 mV were 1·06–1·45 msec in the experiment of Fig. 8 (15·5 °C), 6·5–8·5 msec in the experiment of Fig. 9 (11 °C) and 3·77 msec in Fig. 1 of Gillespie & Meves (1978) (16 °C). Time constants of several msec were also observed for the tails associated with repolarization to the holding potential (−67 to −73 mV) at the end of a 10 or 12 msec depolarizing pulse (Figs. 3, 9 and 11). These slow tails may reflect the slow turning off of the maintained conductance. It should, however, be noted that the very strong venom effect in Fig. 1 is accompanied by a relatively weak effect on the tails (half-time 0·2 msec,

see p. 481); also Fig. 5*A* shows no long-lasting tail. We cannot exclude the possibility that the long-lasting tails associated with repolarization to the holding potential (which have not been observed by Narahashi *et al.* 1972) constitute a side effect of some venom samples.

Chandler & Meves (1970*a*, *b*) assumed that inactivated peak conductance is converted into maintained conductance according to the reaction

$$h_1 \rightleftharpoons \text{inactive} \rightleftharpoons h_2.$$

A possible assumption is that scorpion venoms simply increase the number of channels which go from the peak conductance state into the maintained conductance state, i.e. favour the transition into the h_2 state. The effect of scorpion venoms would then be similar to the effect of replacing the intracellular KF by NaF which according to Chandler & Meves (1970*b*) increases α_{h_2}/β_{h_2} (and therefore $h_{2\infty}$) by a factor of 2 (see also p. 488). On the basis of the h_1–h_2 model, trace *a* of Fig. 9 would be interpreted as a decrease of h_1 (decrease of peak conductance) and a subsequent increase of h_2 (increase of maintained conductance).

It is consistent with this hypothesis that many of the phenomena seen after application of the venoms occur also in untreated axons, but in less pronounced form: a crossing over of the traces is indicated in the control records in Fig. 2*A*, an increase of h_∞ at positive potentials is shown by the control measurements in Figs. 6 and 7 (cf. Fig. 11 of Chandler & Meves, 1970*b*), tail currents following long pulses in untreated axons become larger in the positive pulse potential range (see Fig. 7 of Bezanilla & Armstrong (1977) and Fig. 1 of Gillespie & Meves (1978)), an increase of the Na current after a strong first pulse (Fig. 12) can be demonstrated in untreated axons.

There are, however, two observations which require additional assumptions. One is the apparent saturation of the maintained conductance at strong positive potentials after treatment with a given concentration of venom (Figs. 10*B* and 12*B*; see also Fig. 1 of Gillespie & Meves (1978)) which is not seen in the untreated axon; it would seem that there is an upper limit for the number of channels which go into the h_2 state. The second observation concerns the kinetics of the maintained conductance. From Fig. 2 of Chandler & Meves (1970*b*) the time constant $1/(\alpha_{h_2}+\beta_{h_2})$ at 0 mV and 0 °C is 1·8 msec, corresponding (with a Q_{10} of 3) to 0·5 msec at 11 °C and 0·3 msec at 16 °C. The time constants for the maintained conductance in the venom-treated axon (see preceding paragraph) are 3–17 times larger. After application of venom, but not before, an increase of I_2 with increasing positive pre-pulse potential can be demonstrated with a gap length of 2·2 msec (p. 495). These findings may be explained by assuming that the reaction inactive $\rightleftharpoons h_2$ is drastically slowed by venom.

An alternative to the h_1–h_2 model is the assumption that peak conductance and maintained conductance reflect two separate populations of Na channels which have the same reversal potential and are both TTX-sensitive but differ in their kinetics. It is difficult to decide between these alternatives. The slow time course of the tails associated with repolarization to the holding potential (see above) is clearly not compatible with m^3h_2 kinetics because, on this basis, the fast shutting-off of m should always predominate.

This can be illustrated by an example. If the tail current is proportional to

$$[\exp(-t/\tau_m)]^3 \exp(-t/\tau_{h_2}) = \exp(-(3\tau_m^{-1}+\tau_{h_2}^{-1})t)$$

and if we assume $\tau_m^{-1} = 9{\cdot}54$ msec^{-1} and $\tau_{h2}^{-1} = 3{\cdot}0$ msec^{-1} at -70 mV and 10 °C (Hodgkin & Huxley, 1952; Chandler & Meves, 1970*b*; $Q_{10} = 3$), the time constant for the decay of the tail current is $1/(3\tau_m^{-1}+\tau_{h_2}^{-1}) = 32$ μsec. If venom reduces $\tau_{h_2}^{-1}$ by a factor of 10, the time constant of the tail would only increase to 35 μsec.

However, as mentioned above, the slow tails at the holding potential were not a completely consistent feature of the venom effect, probably due to the variability in the constituents of the crude venoms (see p. 495). In conclusion the question whether the large maintained conductance seen in venom-treated axons is converted peak conductance (as assumed by the h_1–h_2 model) or reflects a separate population of channels must be left open.

Note added in proof. G. N. Mozhayeva, A. P. Naumov, E. D. Nosyreva and E. V. Grishin (*Biochim. biophys. Acta* **597**, 587–602, 1980) have recently studied the effect of purified toxin from venom of the scorpion *Buthus eupeus* on frog myelinated nerve fibres. They found that the h_∞ curve of poisoned fibres rises at $V > -30$ to -40 mV and saturates at positive potentials. In the majority of the experiments there was a slowing of the tail currents during repolarization.

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