Incomplete Sodium Inactivation in Nodes of Ranvier Treated with Scorpion Venom[1]

The action potential of single nodes of Ranvier is markedly prolonged by addition of tetraethylammonium or nickel chloride to the outside medium ([2] and [3]). The most potent substance in this respect seems to be scorpion venom; it lengthens the nodal action potential by a factor of more than 10,000 at 20 °C[4]. The shape of the action potential closely resembles that recorded in Purkinje and ventricular fibres. Analysis of the nodal membrane currents may therefore provide a model for the generation of long-lasting action potentials in excitable tissues. Previous experiments suggested that scorpion venom mainly affects the inactivation of the sodium permeability[4,5]. This paper deals with the time and voltage dependence of the sodium inactivation in nodes of Ranvier treated with scorpion venom.

Single nerve fibres of *Xenopus laevis* were investigated by the voltage clamp technique of DODGE and FRANKENHAEUSER[6]. Experiments were carried out with the experimental arrangement described by KOPPENHÖFER[7]. The node under investigation was continuously superfused with Ringer solution (composition m*M*): KCl 2.5; NaCl 112.9; $NaHCO_3$ 2.4; $CaCl_2$ 1.8. Measurements of membrane currents were started 6 min after addition of scorpion venom (10^{-6} g/ml) to the outside solution. Between pulses the membrane was held at the normal resting potential (V = 0).

If a normal node is depolarized to V = 50 mV, a transient inward sodium current is recorded which is followed by an outward potassium current (Figure 1A). After application of the venom, the inward current decays more slowly (Figure 1B), and a sustained component is observed for 100 msec (Figure 1C) or more. The peak inward current is slightly reduced. Substitution of *Tris* for sodium ions (Figure 1D) or addition of tetrodotoxin (10^{-7} g/ml) abolishes both the transient and maintained components of the inward current. An outward current remains which consists of an unchanged leak current and a reduced potassium current. These experiments show that the sustained inward current is carried by sodium ions.

In order to measure the time course of the sodium permeability P_{Na} during a long-lasting depolarization, the node was superfused with Ringer solution which contained 114.5 m*M* KCl in addition[8]. As usual the membrane potential was clamped at its normal value (V = 0). A test pulse of V = 67 mV depolarized the membrane to the potassium equilibrium potential. The membrane current during the pulse consisted of a leak and a sodium current only; from the latter the time course of P_{Na} was calculated. Filled and open circles in Figure 2 are P_{Na} values measured before (A) and after (B) application of the venom. While the rate of increase of P_{Na} is hardly changed by the venom, the rate of decrease is considerably reduced. This is mainly due to a very slow phase of inactivation (Figure 2B, lower record).

In the normal node, the time course of P_{Na} during a depolarization of more than 30 mV is described by the following equation[9]:

$$P_{Na} = \bar{P}_{Na} \times m_\infty^2 \times (1 - e^{-t/\tau_m})^2 \times h_0 \times e^{-t/\tau_h} \qquad (1)$$

$\bar{P}_{Na} \times m^2_\infty \times h_0$, τ_m and τ_h can be determined experimentally; in the experiment of Figure 2A they were found to be 4.79×10^{-3} cm/sec, 0.03 msec and 0.43 msec, respectively. The continuous lines calculated from equation (1) satisfactorily describe the measured time course of P_{Na}.

Two assumptions were made in order to describe the time course of P_{Na} of the poisoned node by an equation similar to (1):

(a) The inactivation of P_{Na} proceeds in 2 phases; the variable h is split into 2 components, x and y, which change with time constants, τ_x and τ_y, from their initial values, x_0 and y_0, to the steady state values, x_∞ and y_∞.

(b) The inactivation of component x is incomplete.

Thus the time course of P_{Na} is given by the following equation:

$$P_{Na} = \bar{P}_{Na} \times m_\infty^2 \times (1 - e^{-t/\tau_m})^2 \times [x_\infty - (x_\infty - x_0) \times e^{-t/\tau_x} + y_0 \times e^{-t/\tau_y}] \qquad (2)$$

[1] This work was supported by the Deutsche Forschungsgemeinschaft, Bad Godesberg.

[2] H. SCHMIDT, Pflügers Arch. ges. Physiol. *282*, 351 (1965).
[3] H. MEVES, Pflügers Arch. ges. Physiol. *278*, 273 (1963).
[4] K. R. ADAM, H. SCHMIDT, R. STÄMPFLI and C. WEISS, Br. J. Pharmac. Chemother. *26*, 666 (1966).
[5] E. KOPPENHÖFER and H. SCHMIDT, Pflügers Arch. ges. Physiol. *291*, R 18 (1966).
[6] F. A. DODGE and B. FRANKENHAEUSER, J. Physiol. *143*, 76 (1958).
[7] E. KOPPENHÖFER, Pflügers Arch. ges. Physiol. *293*, 34 (1967).
[8] B. FRANKENHAEUSER, J. Physiol. *151*, 491 (1960).
[9] A. L. HODGKIN and A. F. HUXLEY, J. Physiol. *117*, 500 (1952).

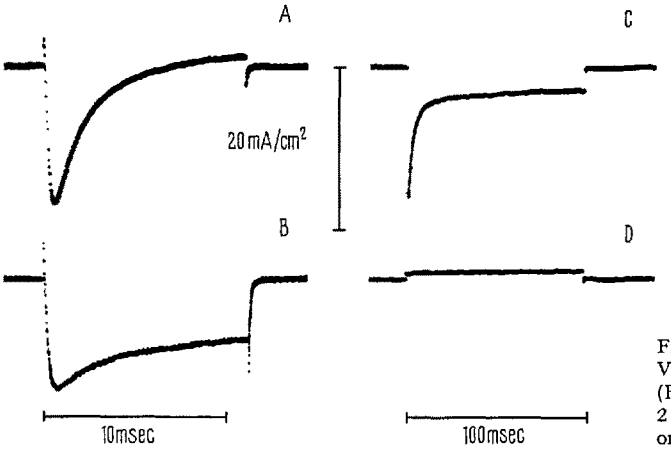

Fig. 1. Membrane currents associated with a depolarizing pulse of V = 50 mV before (A) and after application of scorpion venom (B — D); in D the sodium concentration was reduced from 113 to 2 m*M* (*Tris*-Cl replacing NaCl). Records A and B were taken from one preparation, and C and D from another. Temperature 18 °C.

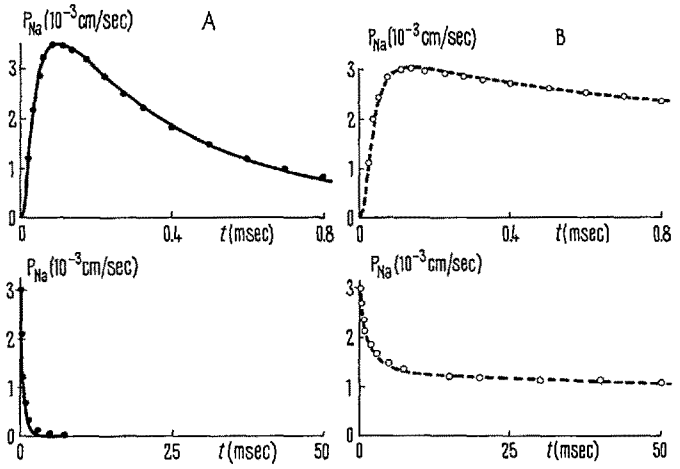

Fig. 2. Time course of the sodium permeability P_{Na} before (A) and after application of scorpion venom (B) to Ringer solution containing 114.5 m*M* KCl in addition. Ordinates: P_{Na} during a depolarizing pulse of V = 67 mV. Abscissae: Duration of pulse. Note different time scales. Filled and open circles are measured values; the continuous and dotted lines were calculated from equations (1) and (2). Temperature 22 °C.

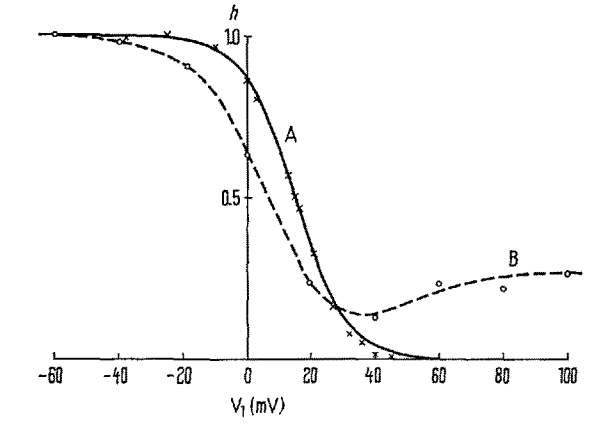

Fig. 3. *h*-V Relation before (crosses) and after application of scorpion venom (circles). Ordinate: Peak P_{Na} during test pulse V_2 = 50 mV given as fractions of the peak P_{Na} which is available at large anodal prepulses V_1. Abscissa: Amplitude of V_1. Curve A calculated by equation (1) given by HODGKIN and HUXLEY[10], curve B drawn by eye. Temperature 18 °C.

In the experiment illustrated by Figure 2B the values of $\bar{P}_{Na} \times m_\infty^2$, $(x_0 + y_0)$ and τ_m were determined to be 3.21×10^{-3} cm/sec, 0.54 and 0.025 msec, respectively. All other values were chosen to give the best fit: $x_0 = 0.435$; $x_\infty = 0.129$; $\tau_x = 1.4$ msec; $y_0 = 0.105$; $\tau_y = 80$ msec. Figure 2B shows that there is reasonable agreement between the measured time course of P_{Na} and the calculated curve (dotted lines).

Incomplete sodium inactivation is shown in Figure 3, where the ability of the membrane to undergo an increase of P_{Na} is given in terms of the variable *h*. The peak value of P_{Na} was determined during a test pulse, V_2, which was preceded by a conditioning prepulse, V_1, of varying amplitude and polarity. The duration of V_1 was 50 msec for the normal node, and 500 msec for the poisoned node. Curve A of Figure 3 represents the h_∞-V relation (inactivation curve) of the normal node. It shows that P_{Na} is fully inactivated at $V_1 > 40$ mV. Scorpion venom (curve B) reduces the *h* value at the normal resting potential (V = 0) to about 75%. The main difference, however, is that *h* remains finite and even has a tendency to increase with depolarizations of more than 40 mV. Curve B does not strictly represent the h_∞-V relation of the poisoned membrane; the difference appears, however, to be small, because prolongation of V_1 from 50 to 500 msec did not change the *h* values by more than 30%. Results similar to those shown in Figure 3 have been found by CHANDLER and MEVES[11] in squid axons internally perfused with NaF solution.

The experiments show that scorpion venom mainly reduces the rate and amount of sodium inactivation. This effect is likely to be responsible for the extreme prolongation of the action potential. The simultaneous reduction of the potassium outward current favours the formation of a plateau during the repolarization phase. Thus, under the influence of scorpion venom, the permeabilities of the nodal membrane behave in a way suggested by several authors[12] to explain the long-lasting action potentials of Purkinje and ventricular fibres on the basis of the Hodgkin-Huxley equations.

A complete description of the results will appear in Pflügers Arch. ges. Physiol.[13].

Zusammenfassung. Voltage-clamp Versuche an markhaltigen Nervenfasern von *Xenopus laevis* ergaben, dass die Inaktivierung der Na-Permeabilität unter dem Einfluss von Skorpiongift extrem verlangsamt und unvollständig ist. Durch Aufspaltung der Variablen *h* der Ionentheorie in 2 voneinander unabhängige Komponenten kann der zeitliche Verlauf der Na-Permeabilität berechnet werden.

E. KOPPENHÖFER and H. SCHMIDT

I. Physiologisches Institut der Universität des Saarlandes, 665 Homburg-Saar (Germany),
24 July 1967.

[10] A. L. HODGKIN and A. F. HUXLEY, J. Physiol. *116*, 497 (1952).
[11] W. K. CHANDLER and H. MEVES, J. Physiol. *186*, 121 P (1966).
[12] S. WEIDMANN, J. Physiol. *115*, 227 (1951). – A. J. BRADY and J. W. WOODBURY, J. Physiol. *154*, 385 (1960). – D. NOBLE, J. Physiol. *160*, 317 (1962). – K. A. DECK and W. TRAUTWEIN, Pflügers Arch. ges. Physiol. *280*, 63 (1964). – R. E. MCALLISTER and D. NOBLE, J. Physiol. *186*, 632 (1966).
[13] We are indebted to Prof. K. R. ADAM, Khartoum, and Dr. C. WEISS, Hamburg, who kindly supplied the scorpion venom.