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Potassium Inactivation in Single Myelinated Nerve Fibres of Xenopus laevis *

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Summary. 1. Voltage clamp measurements were performed on single myelinated nerve fibres of the frog Xenopus laevis.

2. During long-lasting depolarizations the potassium current decayed in a fast phase with a time constant of about 0.6 sec and a following slow phase with a time constant between 3.6 ($V = 0$) and 20 sec ($V = 100$ mV).

3. The decay of the potassium current was the result of an inactivation of the potassium permeability and not of a shift of the potassium equilibrium potential as shown by experiments in isotonic KCl solution.

4. At a hyperpolarization of -20 mV the potassium inactivation was fully removed. It remained incomplete even at large depolarizations. The steady-state inactivation curve was S-shaped but not symmetrical.

5. The experimental results could be described by extending the Hodgkin-Huxley equations introducing two terms of potassium inactivation.

Key-Words: Potassium Inactivation — Voltage Clamp — Ranvier Node.

In various preparations the potassium current slowly decays if the excitable membrane is kept depolarized. This decay has been ascribed to a slow inactivation of the potassium permeability which was found in squid axons (Ehrenstein and Gilbert, 1966; Armstrong, 1969), in nerve cells of snails (Hagiwara *et al.*, 1961; Alving, 1969; Leicht *et al.*, 1971; Neher and Lux, 1971) and in supramedullary cells of the puffer fish (Nakajima and Kusano, 1966). Similar results have been obtained in experiments on electroplaques (Nakamura *et al.*, 1965; Bennett and Grundfest, 1966), Purkinje fibres of dog and sheep (Hall *et al.*, 1963; Hecht *et al.*, 1964) and striated muscle fibres of the frog (Adrian *et al.*, 1970a, b; Kao and Stanfield, 1968, 1970; Stanfield, 1970).

In frog nerve fibres Frankenhaeuser and Waltman (1959) have shown that the membrane resistance increases during long-lasting depolarizations. These authors mentioned that in voltage clamp experiments the potassium current decreased and they interpreted their findings as an inactivation of the potassium permeability. Lüttgau (1960) has drawn

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the same conclusion from current clamp experiments on isolated frog nerve fibres. For the same preparation Moore (1967) reported a clear dependence of the maximum K current on the holding potential suggestive of a slow K inactivation process.

Frankenhaeuser (1963) introduced the variable k for the potassium inactivation but did not present a detailed analysis. Therefore it seemed worthwhile to do voltage clamp experiments on frog nerve fibres to study the kinetics of this inactivation and its voltage-dependence as well as to obtain unequivocal proof that the observed phenomena were indeed caused by changes of the K permeability.

Preliminary reports on this work have appeared (Schwarz and Vogel, 1970, 1971).

Methods

The clawed frogs, Xenopus laevis, were kept at constant conditions (20° C, 14 hour-day and weekly feeding). Single fibres (mean diameter 19 μm) were isolated from the tibial nerve. The fibre was mounted in a perspex chamber. The investigated node of Ranvier was constantly superfused, the nodes on either side were kept in isotonic KCl solution and cut. The membrane currents were recorded with the voltage clamp technique of Dodge and Frankenhaeuser (1958) in a setup described by Koppenhöfer (1967). The current density was calculated from the recorded output voltage of the clamp amplifier by multiplication with $1/(14\ \Omega\text{cm}^2)$ in accordance with Frankenhaeuser (1962a). At the beginning of each experiment the holding potential was determined at which h_∞ was 0.7. This potential was defined as the resting potential and the membrane potential, V, is given as displacement from this value. Depolarizations and outward currents are positive. The leakage current was calculated from anodal impulses assuming that this current was a linear function of membrane potential and did not change with time. The temperature in the experiments was kept at 21° C if not noted otherwise.

The solutions had the following composition (in mM): a) Ringer solution: 110.5 NaCl, 2.5 KCl, 2.4 $NaHCO_3$, 1.8 $CaCl_2$, pH = 7.1 to 8.0; b) isotonic KCl solution: 115.2 KCl, 1.8 $CaCl_2$, pH = 7.5.

Results

The Effect and After-Effect of Sustained Depolarizations

The membrane behaviour during and after long-lasting depolarizations was studied with the impulse program of Fig. 1 (upper row). After a 5-sec hyperpolarization to −20 mV (V_1) cathodal pulses of 37 to 50 sec duration (V_2) were applied yielding the membrane currents that are shown in the lefthand part of this figure. The common feature of records A to D is that during a sustained depolarization the delayed outward current declined from a peak value to a stationary value within 10 to 30 sec depending on V_2. With large depolarizations as in A and B (V_2 = 80 mV) this decay proceeded in two distinct phases while with weaker cathodal pulses as in D the first relatively rapid phase was nearly missing. This rapid phase appeared to be more vulnerable than the slow compo-

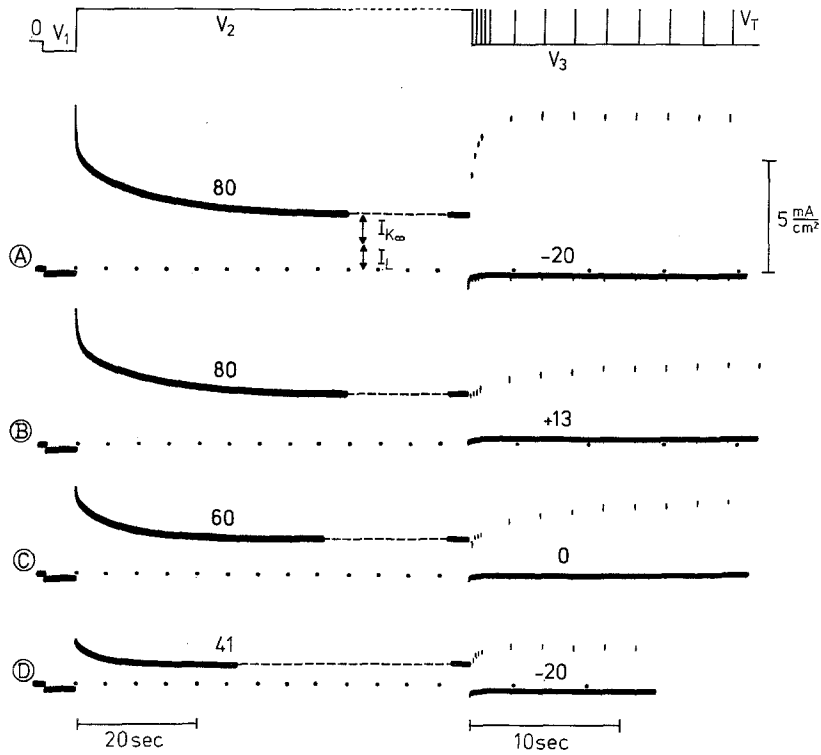

Fig. 1. Effect and after-effect of long-lasting depolarizations. Upper line: impulse program. V_1, conditioning prepulse of −20 mV and 5 sec duration for each record. Potentials V_2 and V_3 are indicated in mV by the numbers attached to the current records. V_T, 30-msec test pulses whose amplitude was identical with respective V_2. $I_{K\infty}$, stationary K current; I_L, leakage current as calculated from anodal impulses. The left-hand part of each record was obtained at a sweep speed of 5 sec/cm which was changed to 2 sec/cm during the fly-back of the free-running beam that subsequently traced the right-hand part. In the figure these records are synchronized on the end of the long pulses although their duration varied: 50 sec in A and B, 46 sec in C, and 37 sec in D. The actual sequence was A-C-D-B. Dots denote the zero current level. 21° C

nent as can be seen by comparing the peak values in records A and B which were obtained with identical pulses V_2 at the beginning and towards the end of the experiment, respectively. The final outward currents in the left-hand part of Fig. 1 were true steady state values, i.e. further prolongation of the cathodal pulses did not change the current amplitude. In one extreme case this was confirmed even for a 10-min depolarization by 80 mV.

The stationary current during a maintained depolarization consisted of two components, a leakage current I_L, and a potassium current, $I_{K\infty}$. As already reported (Schwarz and Vogel, 1971) the latter component was identified by applying 10 mM tetraethylammonium chloride which completely and specificly blocked the K permeability (Hille, 1967; Koppenhöfer, 1967). Hence one may conclude that the observed decline of the outward current during a long-lasting depolarization was exclusively caused by a decay of the K component to a stationary finite level.

The after-effect of long-lasting cathodal pulses is shown in the right-hand part of Fig. 1. The membrane was repolarized to V_3 and a series of short (30 msec) test pulses, V_T (= V_2) was applied. The associated outward currents increased with increasing time after the end of the conditioning pulse (V_2). In records A and D, V_3 was -20 mV and the test currents attained the amplitude of the peak current (at the beginning of V_2) within 2.5 sec. For more positive values of V_3 as in B and C, the membrane recovered more slowly and only partially as judged from the amplitude of the last test current in comparison to the peak current during V_2.

Inactivation of the K Permeability

The decay of the outward current as described in the preceding chapter was observed in various other preparations and commonly interpreted as being caused by an inactivation of the K permeability (see Introduction). Before adopting this interpretation other possible mechanisms had to be excluded. The most obvious alternative was that the sizable K current drawn during a long cathodal pulse might lead to an accumulation of K ions outside the fibre or, more likely, to a depletion inside the axon. In either case a shift of the K equilibrium potential, V_K, would result thereby reducing the driving force for this ion species. To test this possibility the node was superfused with isotonic KCl solution and clamped to a potential near the new V_K. In this way the membrane was depolarized while no net K current could flow to change the concentration of this ion on either side of the membrane. Any change in the K permeability, however, could be detected from a change in the inward K current (tail) that appeared on a short repolarization. The result of such an experiment is shown in Fig. 2. The membrane was first kept at the normal resting potential ($V = 0$) for at least 1 min and then depolarized to $V = 69$ mV which was close to the new V_K as estimated from a current voltage curve. Every second this cathodal pulse was switched off for 7 msec. The amplitude of the K inward currents associated with these repolarizations is plotted in Fig. 2. This quantity decreased exponentially to a stationary value of -1.6 mA/cm^2 with a time constant of 7.7 sec. The tail current amplitude extrapolated

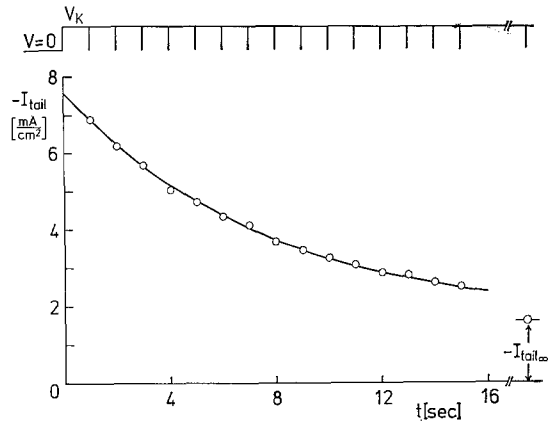

Fig. 2. Inactivation of the potassium permeability in isotonic KCl solution. Ordinate, peak values of the inward tail currents measured during short repolarizations as shown by the impulse program. Abscissa, time after depolarization from the normal resting potential ($V = 0$) to $V = 69$ mV ($\simeq V_K$). o, measuring points. The peak values of I_{tail} decayed to the steady-state value, $I_{tail\infty}$, measured after 30 sec. The continuous curve is calculated from a simple exponential function with a time constant of 7.7 sec. 21° C

to $t = 0$ (-7.6 mA/cm^2) was in good agreement with the results of Frankenhaeuser (1962b) and Koppenhöfer (1967).

In this experiment no change in the driving force for K ions could have occurred. Hence the reduction of the inward current tails must have been due to a decrease of the K permeability. The time constant of this process as observed in this and two other experiments is marked by crosses in Fig. 5. It agreed with the time constant with which—at the same potential—the outward current declined in normal Ringer solution. The obvious conclusion is that, independent of $[K]_0$, the K permeability undergoes an incomplete inactivation when the membrane is kept depolarized.

Steady-State Inactivation

For a quantitative description of the steady-state inactivation, $I_{K\infty}$ attained during a long cathodal pulse was normalized to the peak K current at the beginning of the pulse that was preceded by a 5-sec hyperpolarization to $V = -20$ mV. In Fig. 1 the right-hand part of the records A and D demonstrated that this hyperpolarization was sufficient to completely remove any inactivation. Hence the ratio $I_{K\infty}/I_{K\,peak}$ corresponded to the stationary active fraction of the K permeability for a given depolarization. In Fig. 3 this ratio is plotted against membrane

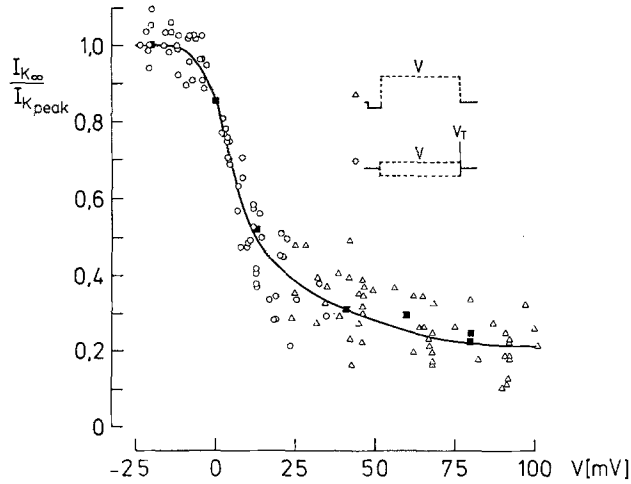

Fig. 3. Steady-state inactivation. Ordinate, stationary potassium current, $I_{K\infty}$, devided by the peak potassium current, $I_{K\,peak}$. Abscissa, membrane potential, V, for which inactivation was determined with the impulse programs shown in the inset: ▵ for large depolarizations, ○ for small displacements from the resting potential. The experimental points from Fig. 1 are denoted ▪. The smooth curve is drawn by eye. 23 fibres

potential for 13 fibres (triangles). The values calculated from the experiment of Fig. 1 were separately marked by filled squares. For $V < 25$ mV the K currents were too small for reliable measurements. Therefore the method illustrated by the lower inset of Fig. 3 was used to determine the steady-state inactivation. This method consisted of measuring the peak K current during a constant test pulse, V_T (= 80 mV, duration 50 msec), following conditioning prepulses of 12.6 sec duration between $V = -20$ and $+25$ mV. The test currents were normalized to those obtained with a prepulse of -20 mV. The normalized values obtained from 9 fibres are given as circles in Fig. 3. Near $V = 30$ mV either method was used yielding comparable results as can be seen from the position of the respective points (triangles and circles).

In our earlier experiments the stationary currents were normalized to those obtained without prepulses ($V = 0$). These results were included in Fig. 3 after correcting for the fact that at the normal resting potential the inactivation was only 86% removed on the average. Therefore some points scatter around 1.0 for $V \simeq -20$ mV. Stronger hyperpolarizing prepulses were also tested confirming the statement that the inactivation was fully removed for $V < -15$ mV. Because of the known damaging effect of lasting strong hyperpolarizations the prepulses were, as a rule, limited to $V \geq -25$ mV.

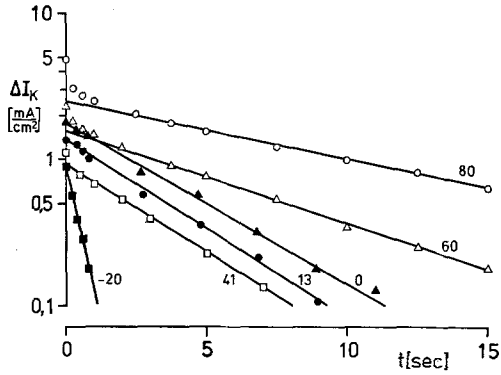

Fig. 4. Semi-logarithmic plot of declining and recovering K currents of Fig. 1. $\Delta I_K = I_K - I_{K\infty}$ for currents during long pulses (open symbols). For recovery measurements (filled symbols) ΔI_K = final test current minus earlier test currents associated with test pulses, V_T, of constant amplitude. The straight lines fit the points for $t > 2$ sec. The numbers attached to the curves refer to the membrane potential in mV. The time constants for the potentials given in brackets are 0.5 (−20), 3.7 (41), 4.2 (13), 4.0 (0), 6.9 (60), and 11.1 sec (80 mV). 21° C

The curve in Fig. 3 resembles that of the Na inactivation (Frankenhaeuser, 1959) in some respects; it is S-shaped and has its maximum slope between $V = 0$ and $V = 10$ mV. In contrast to the curve for the Na system, the curve of Fig. 3 is not symmetrical and the K inactivation remains incomplete ($I_{K\infty}/I_{K\,peak}$ about 0.2) even at large depolarizations.

Time Constants

In Fig. 1 it was shown that during a maintained cathodal polarization the outward current, i.e. its K component, declined in two phases. This diphasic time course is most clearly demonstrated in a semi-logarithmic plot of ΔI_K ($= I_K - I_{K\infty}$) against time. In Fig. 4 such a plot is shown in which the open symbols refer to current values taken from the left-hand records of Fig. 1. Only for $t > 2$ sec could the points be fitted by a straight line whose slope corresponded to the second slow time constant, τ_{k2}. The deviating earlier points indicated another exponential process whose time constant was designated τ_{k1} and which was more pronounced at large depolarizations. While τ_{k2} considerably increased with increasing cathodal polarization, τ_{k1} was not clearly dependent on membrane potential and varied between 0.2 and 1.1 sec at 21° C. Its mean value $\pm$ S.E. was 0.6 $\pm$ 0.07 sec (15 measurements on 5 fibres).

The filled symbols in Fig. 4 refer to the recovery measurements of the right-hand part of Fig. 1. Here, ΔI_K is the difference between the final

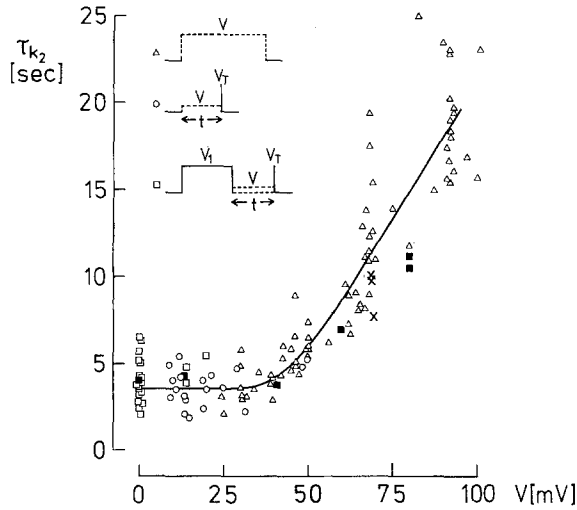

Fig. 5. Time constants, τ_{k2}, of the slow phase of inactivation and its removal versus membrane potential, V. The time constants were determined with the impulse programs shown in the inset: ▵ with strong depolarizations; ○ with short test pulses, V_T, at variable times after small displacements, V, of membrane potential; □ with short test pulses at variable times after repolarization to V to study recovery from inactivation at V_1. Points ■ were taken from Fig. 4, × from experiments in isotonic KCl solution. 46 fibres at 21° C

and earlier test currents. The numbers give the potential level, V_3, from which the constant test pulses arose. Obviously the recovery from inactivation proceeded with a single time constant whose value for $V = -20$ mV was of the order of τ_{k1}.

Fig. 5 gives a survey of the time constants determined in 46 fibres at 21° C. Triangles are values of τ_{k2} during long-lasting depolarizations, circles and hollow squares give the time constants measured with the test pulse programs shown by the respective insets. Independent of the method the average time constant between $V = 0$ and $V = 30$ mV was 3.6 sec. For $V > 30$ mV the time constant increased linearly with depolarization and was about 20 sec at $V = 100$ mV.

The temperature dependence of τ_{k2} for $V = 45$ to 90 mV was tested in 6 experiments by varying the temperature in several steps between 6 and 21° C. An average Q_{10} of $1/\tau_{k2}$ of 2.8 ± 0.1 was found which was close to the value of 2.6 reported by Ehrenstein and Gilbert (1966) for the squid axon. It is interesting to note that from theoretical considerations (Tsien and Noble, 1969) a higher Q_{10} should be expected for a membrane process of very slow kinetics.

Formal Description of Potassium Inactivation

For a quantitative description of the results we applied the equations of Hodgkin and Huxley (1952) and Frankenhaeuser (1963):

$$P_K = \bar{P}_K n^2 k \tag{1}$$

where $\bar{P}_K$ is a constant with the dimension of a permeability which has the numerical value that P_K, the potassium permeability, would take if $n = 1$ and $k = 1$; n and k are dimensionless variables of activation and inactivation, respectively, which can vary between 0 and 1. During a long lasting impulse n will soon attain its final value, n_∞, and we assume that it will stay constant. Hence any change in P_K should reflect the change of k similar to the term h of the Na permeability:

$$k = k_\infty - (k_\infty - k_0)\, e^{-t/\tau_k} \tag{2}$$

where k_0 and k_∞ are the initial and final values, respectively, and τ_k is the time constant of this change.

In order to take care of the fact that there are two phases of inactivation, k was assumed to be the sum of 2 variables.

$$k = k_1 + k_2 \tag{3}$$

where the indeces 1 and 2 denote the fast and the slowly changing component, respectively. Introducing Eq. (3) into Eq. (2) yields

$$k = k_{1\infty} - (k_{1\infty} - k_{10})\, e^{-t/\tau_{k1}} + k_{2\infty} - (k_{2\infty} - k_{20})\, e^{-t/\tau_{k2}}. \tag{4}$$

For three experiments the separation of the steady-state components $k_{1\infty}$ and $k_{2\infty}$ was attempted numerically; the mean result is shown in Fig. 6.

To obtain the numerical values of k the potassium currents recorded during a cathodal pulse were divided by $I_{K\,peak}$ that was observed with this pulse following a hyperpolarizing prepulse of −20 mV. As outlined before, this procedure completely removed the inactivation so that the peak current corresponded to $k = 1.0$. Extrapolating the straight lines in Fig. 4 to $t = 0$ yielded the portion of k that slowly changed from k_{20} to $k_{2\infty}$, i.e. $k_{20} - k_{2\infty}$. This quantity was found to reach a maximum for $V = 40$ mV and it was assumed that for this potential $k_{2\infty} = 0$. This somewhat arbitrary procedure yielded k_{20} that was constant for all potential steps starting from the standard hyperpolarization. As $k_{20} - k_\infty$ was known for various cathodal pulses, $k_{2\infty}$ could be calculated. The stationary value of the fast component, $k_{1\infty}$, was subsequently obtained from Eq. (3): $k_{1\infty} = k_\infty - k_{2\infty}$.

In Fig. 6 the points for k_∞ (filled triangles) are the mean values from the three experiments. The continuous line corresponds to the curve in Fig. 3. The fast component $k_{1\infty}$ (circles) is constant for $V < 40$ mV and decreases for larger depolarizations. The curve of the slow component $k_{2\infty}$ (squares) is nearly symmetrical and S-shaped as is the corresponding

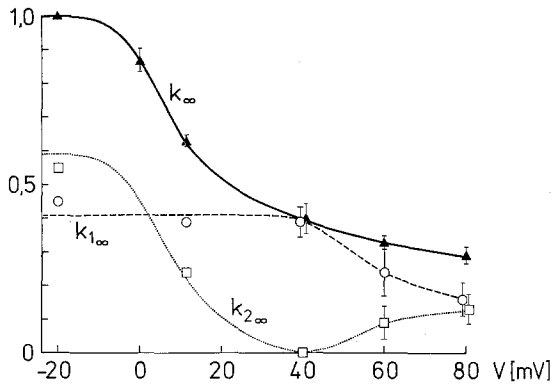

Fig. 6. Stationary relation to membrane potential of the two inactivating components. Ordinate, steady-state availability, k_∞, of the potassium permeability being the sum of $k_{1\infty}$ (fast component) and $k_{2\infty}$ (slow component). Abscissa, displacement of membrane potential, V, from resting potential. Mean values $\pm$ S.E. from 3 experiments calculated as described in text. Overlapping symbols (for $V = 40$ and 80 mV) were separated for clarity. S.E. omitted if smaller than corresponding symbol. The curves were drawn by eye

curve of h (Frankenhaeuser, 1959). At $V \simeq 8$ mV $k_{2\infty}$ reaches 50% of its maximum value. For $V > 40$ mV, $k_{2\infty}$ increases again. Two components of inactivation were also described for the Na system in squid axons internally perfused with NaF (Chandler and Meves, 1970a) and for frog nodes poisoned with scorpion venom (Koppenhöfer and Schmidt, 1968). In either case one of the two components increased again at larger depolarizations.

Discussion

The experiments described in the present paper show that in myelinated nerve fibres the potassium permeability inactivates during long cathodal pulses. For large depolarizations the permeability declines in a fast and a slow phase possibly suggesting two types of K channels. The curve relating the steady-state inactivation to membrane potential is sigmoid; inactivation is incomplete even for $V = 100$ mV.

Lüttgau (1960) reported that in frog nerve fibres depolarized by isotonic KCl solution the membrane resistance increased due to K inactivation with a half time of 15 sec. This value is of the same order of magnitude as the time constant of the slow phase of inactivation observed in the present study at large depolarizations. In desheathed nerve bundles Lewis (1971) recently found comparable time constants of 5.1 sec ($V = 30$ mV) and 6.9 sec ($V = 50$ mV), probably measured at room

temperature. The results of Moore (1967) who described the influence of 3 different holding potentials on the peak potassium current fit well into our inactivation curve.

Our experimental data agree with those observed in squid axons (Ehrenstein and Gilbert, 1966) where K inactivation was also incomplete and described by an S-shaped curve. For an absolute membrane potential of $E = -3$ mV the squid time constant was 33 sec at 9°C which approximately corresponds to our mean value of 12 sec for $V = 70$ mV and 21°C (Q_{10} of 2.6 for the squid membrane). In snail neurons (Leicht *et al.*, 1971) and those of puffer fish (Nakajima and Kusano, 1966) comparable inactivation curves have been found. In the latter preparation the time course of the inactivation was diphasic as it has been described for frog muscle (Stanfield, 1970; Adrian *et al.*, 1970b) whereby the fast component was shown to inactivate completely (Adrian *et al.*, 1970a).

If compared to the sodium system (Frankenhaeuser, 1959, 1960) the inactivation of the potassium permeability essentially differs in four points: 1) the ratio of the time constants of inactivation and activation, τ_k/τ_n is of the order of 10^3 while τ_h/τ_m is about 10, 2) inactivation proceeds in two phases, 3) there is no maximum in the τ_k-V curve, 4) inactivation remains incomplete even at strong depolarizations. On the other hand either inactivation curve is S-shaped. The curve described in the present paper has its maximum slope—predominantly due to the slow component—around $V = 5$ mV. In this region k_∞ decreases by 35% for a 10 mV-depolarization. The mean results of Frankenhaeuser (1959) show a 45% reduction of h_∞ for the same voltage step around $V = 9$ mV.

Because of its slow kinetics the K inactivation does not play a role in the mechanism of the normal action potential. In the socalled 'K action potentials' (Mueller, 1958; Lüttgau, 1960), however, this inactivation process most probably accounts for the slow phase of repolarization.

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