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ULTRA-SLOW INACTIVATION OF THE IONIC CURRENTS THROUGH THE MEMBRANE OF MYELINATED NERVE*

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SUMMARY

(1) Voltage-clamp experiments were performed with myelinated fibres isolated from the sciatic nerve of the frog to study slow changes of the specific sodium and potassium currents as a function of membrane (holding) potential and time.

(2) The level of the peak sodium current depends on holding potential V_H. This dependence can be described by a sigmoidal function $u_\infty(V_H)$. The underlying process is called "ultra-slow sodium inactivation" and is different and separable from the short time steady-state inactivation, $h_\infty(V)$, and from the slow inactivation depending on the extracellular potassium concentration (Adelman, Jr., W. J. and Palti, Y. (1969), J. Gen. Physiol. 54, 589–606; Peganov, E. M., Khodorov, B. I. and Shishkova, L. D. (1973), Bull. Exp. Biol. Med. 25, 15–19; Khodorov, B. I., Shishkova, L. D. and Peganov, E. M. (1974), Bull. Exp. Biol. Med. 3, 10–14).

(3) After a sudden change of the holding potential the sodium current reaches a new steady-state level (due to the transition of $u_\infty(V_H)$ to the corresponding value) within approx. 4 min. The kinetics of the transition cannot be described by a single exponential function.

(4) A corresponding voltage- and time-dependent process of ultra-slow inactivation exists for the potassium current in the node of Ranvier. The kinetics are faster than those of the sodium system.

INTRODUCTION

The level of the peak inward sodium current in voltage-clamped nodes of Ranvier depends on a conditioning membrane potential [1]. Frankenhaeuser [2] described this voltage dependence in 1960 by a "sodium inactivation" function $h(V, t)$ in accordance with the Hodgkin and Huxley formalism for the giant axon of the squid [3]. He demonstrated that the h function reaches different steady-state levels within less than 30 ms. In 1964 Narahashi [4] described the effect of the outside K^+ concentration on the level of the peak values of the transient sodium current

* Dedicated to Professor Dr. Hermann Muth on the occasion of his 60th birthday.

in the lobster giant axon, which had a time constant approaching 1 s. The effect was studied in detail in the squid axon by Adelman and Palti [5] and was attributed to a possible depletion of K^+ in the Frankenhaeuser-Hodgkin space [6] by a long-lasting hyperpolarization. The process was mathematically described by entering a function $p(V, t)$ into the Hodgkin-Huxley equation for the sodium conductance. $p(V, t)$ varies slowly in a sigmoidal manner with V and exponentially with time, like $h(V, t)$, but with a time constant of approx. 300 ms. A similar phenomenon was observed by Chandler and Meves [7] in squid giant axons perfused with NaF.

This "slow" sodium inactivation process, depending on the outside K^+ concentration, was shown to exist also in the node of Ranvier by Peganov et al. [8]. It was named the s function, with properties similar to the p function used in the squid giant axon. The variation of $s(V, t)$ with the external Ca^{2+} concentration and the influence of procaine were studied by Khodorov et al. [9, 10].

Adelman and Palti [5] detected, in addition, an "ultra-slow" sodium inactivation process in the squid giant axon, also of a double sigmoidal voltage dependence, but with a time constant in the range of 30–200 s. This mechanism, named q, was shown to be independent of the external K^+ concentration, but further properties were not measured and no hypothesis for its explanation was proposed.

The objective of this paper is, therefore, to investigate whether such an ultra-slow inactivation process exists in myelinated nerves and to describe its properties.

METHODS

(1) *Measurement of currents*

Motor and sensory fibres isolated from the sciatic nerve of the frog *Rana esculenta* were investigated under current- and voltage-clamp conditions using the technique developed by Nonner [11]. At the beginning of each experiment the Ringer's solution in the side pools (pools C and E) was replaced by isotonic KCl solution or by an artificial axoplasm (see below). Then the internodes on either side of the node under investigation were cut near the neighbouring nodes (in pools C and E).

The holding potential was set to approximately resting potential (no negative after potential in current-clamp; $h_\infty = 0.65$–0.70 in voltage-clamp). The baseline of current at holding potential was continuously recorded with an ink writer. After an initial stabilization period of about 15 min the DC balance of the voltage-clamp system did not require readjustments. Afterwards the drift of the holding current did not exceed 0.2 nA/h in good experiments, corresponding to approx. 3 mV/h change in holding potential. (The calibration of the holding current in terms of holding potential was achieved by measuring the holding current before and after application of a test change of the holding potential.) The accuracy of determination of the holding current was ± 0.02 nA.

The experiments were performed on-line with a processing computer (Honeywell DDP-516), which was used to sample and digitalize the analogue signals from the output of the voltage-clamp amplifier with the aid of an analogue to digital converter (Raytheon, resolution 10 bit; aperture time 100 ns; throughput rate 50 kcycles/s; accuracy 0.1 % of full range). Sequences (see below) of voltage pulses

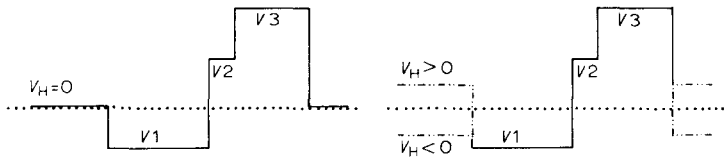

Fig. 1. Sequence of voltage pulses and holding potential as applied to the nodal membrane in voltage-clamp.

were applied to the preparation from the computer using a digital to analogue converter (Honeywell, resolution 10 bit; accuracy ± 0.1 %; settling time 8 μs). The experimental programs were written in a special macroassembler language [12] providing the necessary instructions for measurements under real time conditions and for digital data processing. The digitalized ionic currents were corrected for leakage and for offset currents of the recording system (both separately determined at certain intervals) and were stored on digital magnetic tape.

The sequence of voltage pulses applied to the nodal membrane in voltage-clamp is illustrated in Fig. 1. The depolarizing short test pulse, $V2$, to measure the early peak inward current, I_{Na}, is preceded by a hyperpolarizing conditioning pulse, $V1$, and followed by a more positive long lasting test pulse, $V3$, to determine the steady-state outward current, I_K. All voltages refer to the baseline given by the initial adjustment of the voltage-clamp system (DC balance). A positive or negative holding potential V_H was applied by setting the analogue computer output to adequate voltage levels between two recording periods without changing the absolute amplitudes of the pulse sequence (see Fig. 1).

The standard settings used throughout the experiments were (if not otherwise indicated):

prepulse	$V1 = -40$ mV;	50 ms
1st test pulse	$V2 = 60$ mV;	1–2 ms
2nd test pulse	$V3 = 120$ mV;	20 ms

To record a shift of the $h_\infty(V)$ curve along the voltage axis, $h_\infty(V = 0)$ was determined by the following procedure. The peak sodium current was measured with the conditioning prepulse $V1$ set to a negative potential sufficiently high to remove sodium inactivation ($h_\infty = 1$). 2 s later the sodium current was measured again but with the prepulse set to $V1 = 0$. Then the ratio $I_{Na}(V1 = 0)/I_{Na}(V1 < 0; h_\infty = 1) = h_\infty(V = 0)$ was evaluated. This procedure of shift measurement is adequate for the case of a mere translation of the $h_\infty(V)$ curve along the voltage axis.

The peak sodium current was determined without correcting for the small portion of I_K being present in the early peak inward current.

(2) *Nomenclature*

The formalism introduced by Frankenhaeuser and Huxley [13] is used to describe the ionic currents.

Potentials are referred to the external solution and are given as $V = E - E_r$. "Hyperpolarization" and "depolarization" have their usual meaning, that is (in the V scale) a negative or a positive deviation from resting potential.

Inward currents, consequently, are negative. For comparison with earlier work the ionic currents were calibrated as current densities as described elsewhere [14]. These current values have to be considered as rough estimates of the true current densities in the nodal membrane [14].

(3) *Solutions*

The Ringer's solution contained 110 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$ and 5 mM tris(hydroxymethyl)aminomethane · HCl buffered at pH 7.3. The low pH Ringer's solutions were buffered with phthalic acid instead of Tris · HCl, using the procedure of Drouin and The [15]. The conductivity of all solutions was within $\pm 5\,\%$ of the value for normal Ringer's solution. The artificial axoplasm solution in contact with the cut ends of the fibres contained 103 mM KCl, 10 mM NaCl and 5 mM $Na_2HPO_4 + KH_2PO_4$ (1: 1) buffer (pH 6.88).

RESULTS

(1) *Effect of membrane potential*

According to the Frankenhaeuser-Huxley theory [13] the peak value of the sodium current should not vary with membrane (holding) potential, if it is determined at a constant test voltage, which is preceded by a conditioning pulse of sufficient duration and amplitude to remove sodium inactivation. Also, the steady-state potassium current should not vary with membrane (holding) potential, if determined at a constant test voltage. However, if the holding potential suddenly is changed from $V_H = 0$ mV to negative values, both the peak sodium and the potassium currents actually increase slowly to new steady levels; the opposite is found if the holding potential is set to positive values: the currents then decrease slowly to new steady levels (Fig. 2).

The experimental conditions (standard settings of voltage pulses, see Fig. 1) should provide maximum permeability of the sodium channels ($m_\infty \approx 1, h_\infty \approx 1$). Nevertheless, at $V_H = -30$ mV the peak sodium inward current is increased beyond the level of complete sodium activation at $V_H = 0$ mV. (The solid lines represent the expected normal exponential decline of the ionic currents (run-down) at $V_H = 0$ during a long lasting voltage-clamp experiment [16] as fitted to the experimental points of the initial period and drawn as a continuation of the current values measured at $V_H = 0$.) Also in contrast to the conditions of complete activation the ionic currents are decreased by a positive holding potential.

After a step to the negative holding potential, the peak sodium current reaches its new steady-state level I_{Na}^{∞} within about 4 min, but I_K^{∞} is reached in less than 1 min. After switching back to zero holding potential, the sodium current declines rapidly during the first 4 min, then more slowly, and would reach the current values expected from the normal exponential decline (solid curves) after about 15 min. The potassium current changes back much more rapidly than the sodium current.

The following investigation of the voltage and time dependence of the effects illustrated in Fig. 2 will be concerned only with the changes of the sodium current. An inactivation process in the sense of the Frankenhaeuser-Huxley theory [13] requires a sigmoidal potential dependence of the degree of inactivation. This was studied by determining the ratio of the maximum available peak sodium current

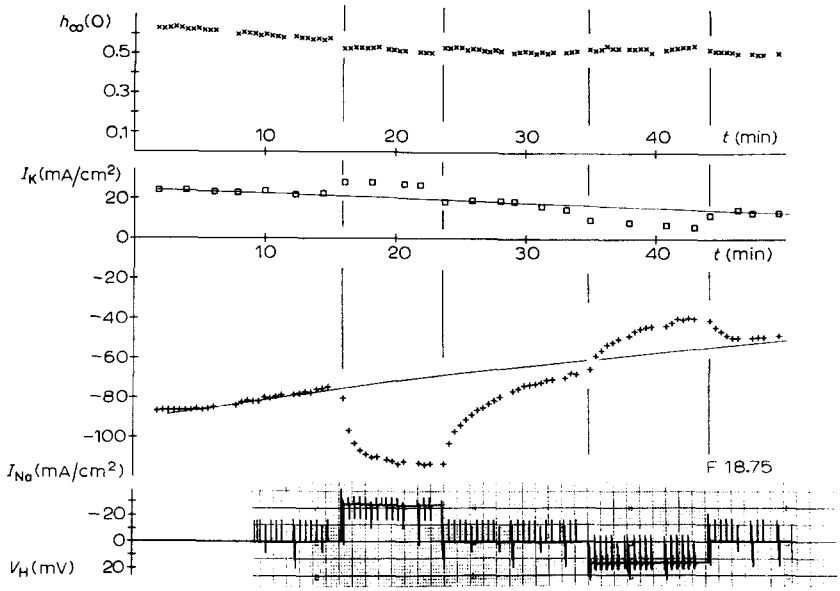

Fig. 2. Effect of negative and positive holding potentials on the level of the nodal ionic currents, I_{Na} (+) and I_K (□), and on $h_\infty(V=0)$ (×). Determination of currents under the conditions given in Fig. 1. The expected normal exponential decline (run-down) of the currents at $V_H = 0$ as fitted from the initial recordings (first 15 min) is represented by the solid curves. The holding current as recorded simultaneously was calibrated in terms of holding potential. In contrast to the experimental conditions of complete sodium activation ($h_\infty \approx 1$, $m_\infty \approx 1$) a negative holding potential causes an increase of the peak sodium current, and a positive holding potential causes a decrease. The transition to new steady current levels is complete after several minutes. The potassium current changes similarly with holding potential, but the transitions to new steady levels are complete in less than 1 min. The value of h_∞ measured at $V = 0$, h_∞ ($V = 0$), is not significantly affected. Temperature 15 °C.

$I^\infty_{Na}(V_H)/I_{Na}(V_H = 0)$ measured after and before a sudden change of the holding potential starting from $V_H = 0$. The data were plotted versus holding potential (Fig. 3).

Because of the exponential decline of I_{Na} (run-down) the ratio $I^\infty_{Na}(V_H)/I_{Na}$ ($V_H = 0$) depends on the time at which the holding potential is changed (see Fig. 2). The measured values, therefore, were related to $t = 0$ (beginning of the experiment). The procedure or correction is given in the Appendix. The steady-state level of the peak sodium current saturates at high hyperpolarizations. Thus, the I_∞/I_0 values can be normalized by taking the saturation level as unity. The voltage dependence of the normalized values was fitted by a sigmoidal function of the holding potential

$$u_\infty(V_H) = \frac{1}{1+\exp\left(\frac{V'-V_H}{k_M}\right)} \tag{1}$$

with a slope factor of $k_M = (-11.7\ \text{mV})$ and a midpoint potential of $V' = 10.5$ mV (see Fig. 3).

The transition of $u(V_H, t)$ to a new steady-state value after a change of V_H is

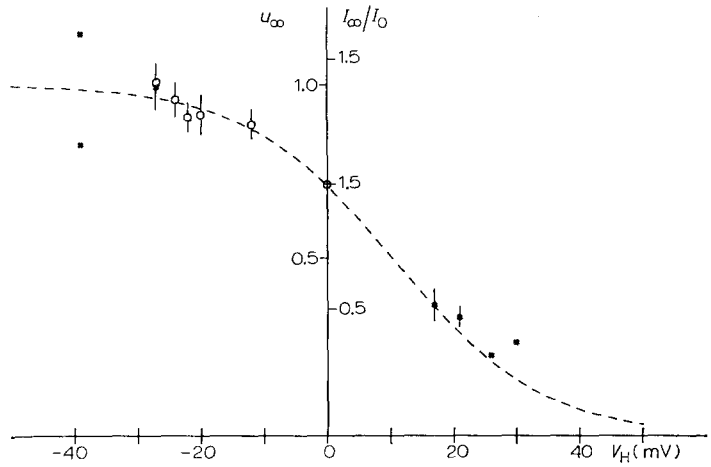

Fig. 3. Voltage dependence of the long-term steady-state levels of the nodal peak sodium current. The peak sodium current was measured at different values of holding potential V_H after reaching the steady state and referred to the current level as measured at $V_H = 0$. The current ratios thus obtained were corrected for exponential decline of the sodium current and plotted versus holding potential (for details see text). Vertical bars indicate the standard deviation from the mean of several different (on the average 4) determinations. Data from 26 different nodes. Temperature 15 °C. (*) Experiments by Schwarz. The normalized experimental points can be fitted by the double sigmoid function of Eqn 1 with a slope factor of −11.7 mV and a midpoint potential of 10.5 mV (broken curve).

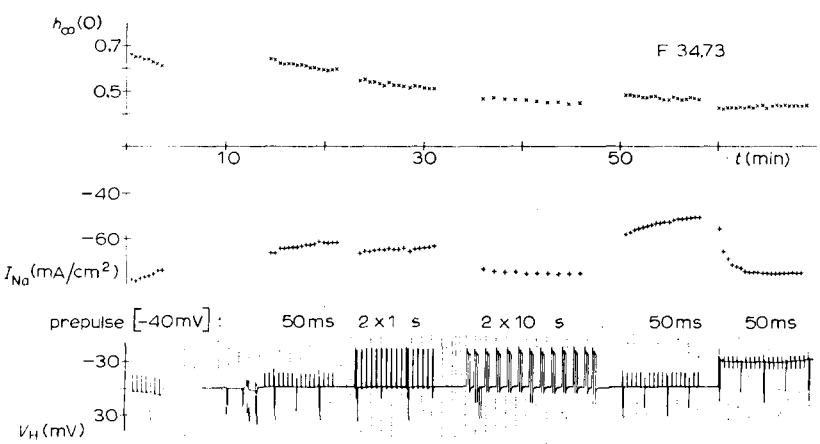

Fig. 4. Influence of prolonged negative prepulses and of a negative holding potential on the nodal peak sodium current, I_{Na} (+), and on h_∞ ($V = 0$) (×). Determination of the variables as described in the Methods (Fig. 1). The ionic currents are increased with increasing duration of the hyperpolarizing prepulse. (The currents were determined as means of two subsequent registrations.) h_∞ ($V = 0$) is not influenced. Temperature 15 °C. At the beginning of the experiment (phase of stabilization of the voltage-clamp system) the influence of an unstable holding potential can be seen: apparent depolarization causes an acceleration of current decline.

a slow process. It might be expected to follow an exponential time course in analogy to the short-term sodium inactivation function $h(V, t)$ used by Frankenhaeuser and Huxley [13]. However, experiments similar to that illustrated in Fig. 4 proved a non-exponential time course. The initially rapid and finally slow increase of the peak sodium current due to a negative step of the holding potential together cannot be described by a single exponential function (Fig. 4). Though the new steady-state level of the sodium current is reached after about 4 min, negative prepulses of 20 s and even of 2 s duration cause a considerable increase of the sodium current, which is incompatible with a single time constant. (Note that the normal sodium inactivation at $V = 0$, $h_\infty(V = 0)$, is altered neither by application of a long prepulse nor by changing the holding potential!)

The time course of the transition of the peak sodium current to a new level after a sudden change of the holding potential can be described by two time constants (Fig. 5). To evaluate this relationship, normalized changes of I_{Na} were introduced by

$$\Delta I(t) = \frac{I_{Na}(t) - I_{Na}^{\infty}}{I_{Na}(0) - I_{Na}^{\infty}} \tag{2}$$

where t is the time after the potential step and I_{Na}^{∞} is the steady level of I_{Na} at the new holding potential. This definition of $\Delta I(t)$ implies the normalization $\Delta I(0) = 1$ for positive and negative changes of the holding potential. $\Delta I(t)$ calculated from the data of the transition shown in Fig. 4 (far right) were plotted semilogarithmically over time (Fig. 5). The experimental points were fitted (solid line) by an equation of the type

$$\Delta I(t) = A \cdot \exp(-t/\tau_1) + B \cdot \exp(-t/\tau_2) \tag{3}$$

Obviously, a single exponential function does not describe the data, but there is a satisfactory fit by two exponentials. Table I summarizes the results of this curve fitting

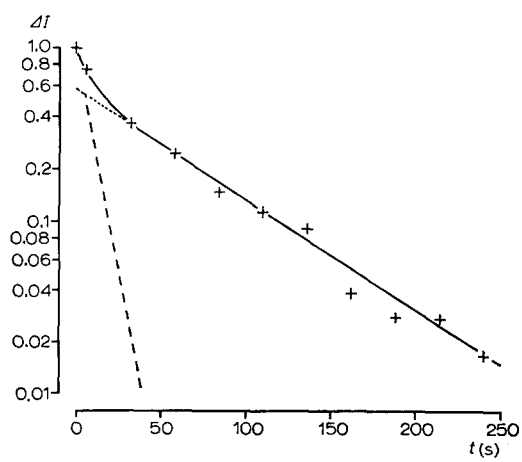

Fig. 5. Time course of the transition of the peak sodium current after a switch of the holding potential from $V_H = 0$ to $V_H = -30$ mV. Data from Fig. 4 (far right) were normalized according to Eqn. 2 and were plotted semilogarithmically versus time. The function of Eqn. 3 was fitted to the normalized experimental points (—). Fast time constant (- - -), slow time constant (· · ·).

TABLE I

TIME CONSTANTS DESCRIBING THE TIME COURSE OF THE TRANSITION OF THE PEAK SODIUM CURRENT AFTER A STEP CHANGE OF THE HOLDING POTENTIAL AS EVALUATED BY FITTING THE FUNCTION OF EQN. 3 TO THE EXPERIMENTAL POINTS

Parameters of Eqn. 3 (mean ±S.D.), n = number of experiments.

Transition of V_H	A	τ_1[s]	B	τ_2[s]	n
0 mV → −30 mV	0.53±0.10	6.6±1.9	0.47±0.10	94±20	5
−30 mV → 0 mV	0.48±0.05	3.6±2.2	0.52±0.05	123±39	4

from different experiments. The parameters A and B of Eqn. 3 are both found to be around 0.5. The time constants τ_1 and τ_2 differ by more than one order of magnitude; the fast time constant τ_1 is more than a factor of 10 longer than the values reported for the slow sodium inactivation [5, 7–10]. The slow time constant τ_2, however, is in the range of the values reported for the ultra-slow sodium inactivation in squid giant axons [5]. There are no significant differences in the parameters obtained with setting or resetting a negative holding potential, respectively.

The changes of the peak sodium current with holding potential at constant conditioning and test potentials could possibly be caused by variations of the ratio τ_m/τ_h of the time constants of sodium activation and inactivation. This possibility is excluded by the finding that the time course of the early sodium current is practically unchanged with holding potential. Fig. 6 shows two traces of the early ionic current (digitalized computer output of the original trace, leak current substracted) taken at $V_H = 0$ and $V_H = -30$ mV. The peak current at $V_H = -30$ mV is greater than at $V_H = 0$ by a factor of 1.4. The current trace at $V_H = -30$ mV reduced by

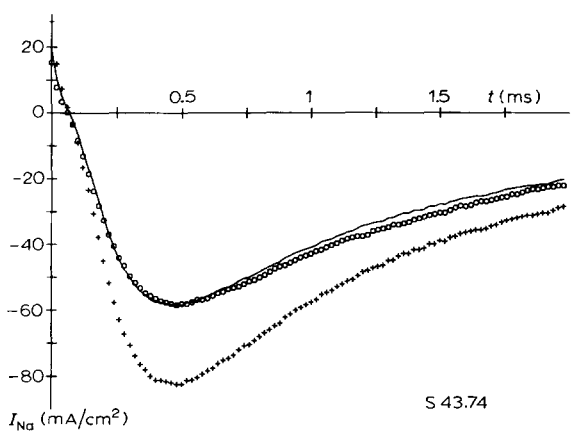

Fig. 6. Time course of the early transient ionic current (digitalized computer output, leak current subtracted) as measured at $V_H = 0$ mV (○) and at $V_H = -30$ mV (+) after reaching the new stationary level. The time between the two measurements is 12 min. The solid line represents the ionic current at $V_H = -30$ mV (+) multiplied by a factor of 0.71. There is no significant change of the time course due to variation of the holding potential. The slight difference in the decline of the inward current can be explained by the variation of the I_{Na}/I_K ratio.

this factor (solid line) overlaps the trace measured at $V_H = 0$ during the first millisecond. (There is a slight difference in the decline after 1 ms, which might be caused by a different ratio of I_{Na}/I_K in both cases.)

(2) *Effect of cations*

Binding of cations to the outer surface of the nerve membrane causes a shift of the kinetic parameters of sodium activation and inactivation along the voltage axis in the positive direction.

It is, therefore, expected that the $u_\infty(V_H)$ curve might also shift in the positive direction with increased extracellular concentration of surface binding cations, for example at lowered extracellular pH. Consequently, an increase of the peak sodium current should be observed owing to removal of ultra-slow sodium inactivation. This was tested in experiments similar to that of Fig. 7. The node of Ranvier was superfused alternately with Ringer's solutions of pH 7.3 and 5.3. During periods of low pH the peak sodium current under the experimental conditions in question (see Fig. 1) is reduced by a partial block of sodium channels (e.g. ref. 17). The initial value of the reduced peak sodium current is not stable, but increases slowly with essentially the same time course to a new stationary value as observed when switching to a more negative holding potential (see especially periods of low pH No. 1, 3 and 6 in Fig. 7). After switching back to pH 7.3 the peak sodium current does not return to the values measured at pH 7.3 before application of the low pH solution, but exceeds this level by approximately 25 %. Since m_∞ and h_∞ are not altered (complete activation) this extra current has to be attributed to an increase of the sodium per-

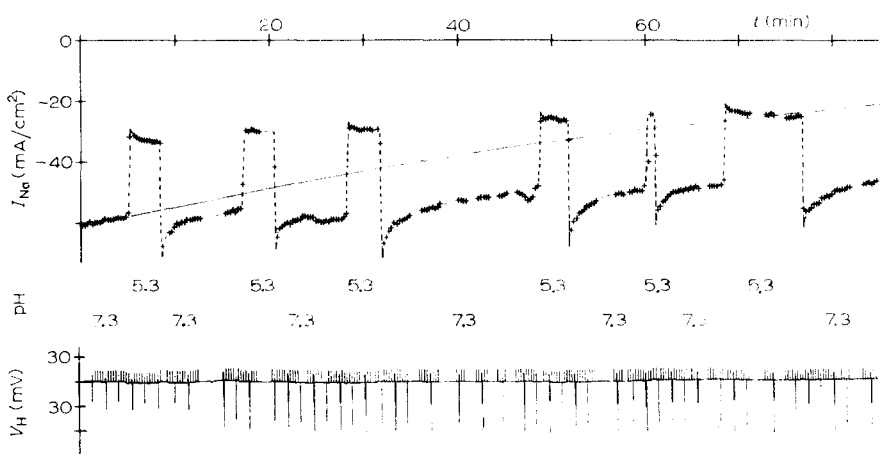

Fig. 7. Long-term changes of the nodal peak sodium current I_{Na} (+), due to repeated periods of lowered pH in the external solution. Determination of currents under the conditions as given in Fig. 1. The pH of the superfusing Ringer's solution was reduced 6 times from 7.3 to 5.3. During the periods of low pH the sodium currents are reduced owing to block of about 40 % of the sodium channels and to shift of the $m_\infty(V)$ curve in the positive direction [18]. At low pH the peak sodium current increases with essentially the same time course as after a negative step of holding potential (Fig. 2). At the return to normal Ringer's solution (pH 7.3) an extra sodium current (+25 %) is observed which decays in a similar way as after the return from a negative to zero holding potential (see Fig. 2.) The amplitude of the extra current does not depend on the duration of the low pH period if not shorter than 3 min (see fifth period of pH 5.3). The holding potential was stable throughout the experiment within ±2 mV. Temperature 15 °C.

meability. This extra current declines with essentially the same time course as seen when switching to a more positive holding potential. The amplitude of the extra current depends only on the duration of the period of lowered pH, if shorter than 3 min (period of low pH No. 5 in Fig. 7). This is in agreement with the observation that the peak sodium current enhanced by a hyperpolarization does not reach its new steady level in periods shorter than 3 min.

Similar observations were made with increased and lowered extracellular concentration of Ca^{2+} (see also ref. 18). Quantitative aspects of the effect of cations on the slow changes of the sodium permeability will be presented in a subsequent paper [19].

DISCUSSION

(1) *Sources of error: effect of series resistance and instability of holding potential*

The voltage drop at a membrane series resistance induces a current-dependent error in the clamp potential. The magnitude of this error has been estimated by Drouin and Neumcke [17]. For preparations of isolated nerve fibres with the internodes cut in the middle (see Methods) the voltage drop at the series resistance is $U(\text{mV}) = 0.074 \cdot I_M$. The current density through the nodal membrane, I_M, is measured in mA/cm^2. If, for instance, the sodium current density is increased from 50 to 100 mA/cm^2 owing to removal of ultra-slow sodium inactivation, the voltage drop is $\Delta U = -3.7$ mV. Since, according to Frankenhaeuser [2], the peak sodium current is a function of the voltage-dependent parameters sodium conductance g_{Na}, driving force $V - V_{Na}$ and ratio τ_m/τ_h of the time constants of activation and inactivation, an error in the clamp potential will retroact to the observed value of I_{Na}. Using the equations of Frankenhaeuser and Huxley [13] this error in I_{Na} can be estimated. In the region of the standard test pulse of $V = 60$ mV used in this work, an increase by 3 mV will reduce the driving force by 4 % and the ratio τ_m/τ_h by 5 %, whereas the sodium conductance is practically constant. The peak sodium current will be reduced under these conditions by 3 %.

In fact, Fig. 6 shows that there is no significant change of the time constants of sodium activation and inactivation due to a negative step of the holding potential. The small differences seen in the decline of the sodium current can be explained by the change of the I_{Na}/I_K ratio.

The possibility that short time changes in holding potential, for instance electrode polarization, cause the transitions of the peak sodium current to new steady levels can be excluded by the facts that the ratio τ_m/τ_h is not altered (see above) and that $h_\infty(V = 0)$ is not greatly affected by a step to a negative or positive holding potential (Fig. 4), meaning that the zero potential level is not significantly changed. A further negative argument is that the rapid reduction of the peak sodium current at low pH is followed by a slow increase of the current, but is not accompanied by any deviation of the holding potential at all (Fig. 7).

(2) *Ultra-slow inactivation of nodal ionic currents*

The existence and phenomenology of a slow process controlling the sodium conductance in the node of Ranvier has been revealed. The sigmoidal dependence of the sodium conductance on holding potential and the long periods necessary for

a transition from one steady-state level to another following a change in potential led to the denotation "ultra-slow sodium inactivation". This process has to be distinguished from the classical short-term sodium inactivation (in the millisecond range) described by Hodgkin and Huxley [3] and from a slow inactivation process in the hundred millisecond range [5, 7–10]. From the observations of the present work the existence of a similar process of "ultra-slow potassium inactivation" in the node of Ranvier is most probable. As detailed properties of this potassium inactivation were not studied, the following discussion will be restricted to the sodium system.

The possible existence of an ultra-slow sodium inactivation in the squid giant axon has been reported by Adelman and Palti [5], but the phenomenon was not investigated in detail. No data at all have been reported so far for the node of Ranvier.

The underlying mechanism of the process is entirely obscure. Its sigmoidal voltage dependence (see Fig. 3) leads to the hypothesis that the distribution of charged components (dipoles or particulated complexes) within the membrane is altered by changes of the electric field strength, assuming that the displacement of charges is directly related to the long-term steady-state ionic conductance. Fitting of Eqn. 1 to the data (Fig. 3) results in the parameter values $V' = 10.5 \pm 2$ mV and $k_M = -11.7 \pm 2.5$ mV. V' is the potential at which the charged components are equally distributed between the conducting and the blocking configurations (midpoint potential), k_M is the slope factor. The corresponding values for the $h_\infty(V)$ curve are $V' = 5.3 \pm 0.6$ mV and $k_M = -7.0 \pm 0.8$ mV [20].

A conclusion with respect to a direct interrelation between the two processes cannot be drawn from the data available. An indication for a possible interaction of the ultra-slow inactivation and the activation mechanism, however, is provided by the observation that the "gating currents" for sodium in squid giant axons increase with decreasing holding potential [21, 22] parallel to the sodium conductance (i.e. possibly with the removal of ultraslow inactivation).

The kinetics of the ultra-slow sodium inactivation have been shown to be non-exponential. They can be described by a set of two exponential functions with two time constants in the range of seconds and minutes, respectively. As has been pointed out already in the Results section, the fast time constant deviates by a factor of 10 from the time constant found for the so-called "slow sodium inactivation" in the squid giant axon and in the node of Ranvier. Consequently, one would have to assume another two processes with exponential time courses to explain the phenomena. A more satisfactory concept, therefore, is one process describing the whole time course of ultra-slow sodium inactivation by one characteristic time. The following paper is concerned with an electrodiffusion mechanism that describes all phenomena presented in this paper.

(3) *Effect of cations*

As was previously reported [18, 23] external application of high-$[Ca^{2+}]$ or high-$[H^+]$ solutions causes an extra sodium current after the return to normal conditions; this extra current decays within a few minutes. Though this phenomenon is a general experience, also occurring with other cations, an explanation has been missing so far. Extracellularly applied Ca^{2+} or H^+ cause an apparent change of the intrinsic field strength across the nodal membrane in the sense of an external hyper-

polarization [17, 23–25]. Consequently, changing the external cation concentration originates a transition of the ultra-slow inactivation to a new stationary level. At low extracellular pH the peak sodium current thus increases slowly with time to a new stationary value. The full extent of this increase does not become visible, since part of the sodium channels are blocked by protons (e.g. refs. 15, 17, 23, 26). The increment of current is fully revealed after the return to normal conditions, but then the electric field strength is suddenly lowered owing to rapid unbinding of the protons and, therefore, the extra current decays to its value normal for $V_H = 0$. The amplitude and time course of these changes are in agreement with the variations of the peak sodium current observed after sudden changes of the holding potential. For quantitative aspects see the following paper.

If this interpretation is correct the ultra-slow inactivation process must be considered as an integral component of the permeability mechanism of the sodium channel, because it controls the sodium permeability dependent on the intrinsic electric field of the membrane.

APPENDIX: PROCEDURE OF CORRECTION OF $u_\infty(V_H)$ FOR EXPONENTIAL DECLINE OF THE SODIUM CURRENT

Because of the decline of the peak sodium current during long-lasting voltage clamp experiments (run-down) the current ratio $I_{Na}^{\infty}(V_H, t)/I_{Na}(V_H = 0)$ is a function of time t after the beginning of the experiments. For comparison of the data from different experiments, therefore, a correction for the exponential decline is necessary. The decline of I_{Na} can be described phenomenologically by a single exponential function with time [16]. From the current measurements two ratios can be obtained: $I_{Na}^{\infty}(V_H, t)/I_{Na}(V_H = 0, t)$ = ratio of I_{Na} after and before change of holding potential at time t, and $I_{Na}(V_H = 0, t)/I_{Na}(V_H = 0, t = 0)$ = ratio of I_{Na} at time t and at the beginning of the experiment. Thus, $I_{Na}^{\infty}(V_H, t)$ can be referred to $I_{Na}(V_H = 0, t = 0)$:

$$\frac{I_\infty}{I_0} = \frac{I_{Na}^{\infty}(V_H, t)}{I_{Na}(V_H = 0, t)} \cdot \frac{I_{Na}(V_H = 0, t)}{I_{Na}(V_H = 0, t = 0)} \tag{4}$$

Since there are no experimental data available for back extrapolation of $I_{Na}^{\infty}(V_H, t)$ to $t = 0$, $I_{Na}^{\infty}(V_H, t)$ is attributed to $V_H + \Delta V(t)$. $\Delta V(t)$ is the observed exponential shift of the $h_\infty(V)$ curve during the time t from the beginning of the experiment, as evaluated from the decrease of $h_\infty(V = 0)$ [16]. The thus obtained values

$$\frac{I_\infty}{I_0} = \frac{I_{Na}^{\infty}(V_H + \Delta V)}{I_{Na}(V_H = 0, t = 0)} \tag{5}$$

were plotted versus $V_H + \Delta V$ in Fig. 4.

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