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KINETICS OF THE SLOW VARIATION OF PEAK SODIUM CURRENT IN THE MEMBRANE OF MYELINATED NERVE FOLLOWING CHANGES OF HOLDING POTENTIAL OR EXTRACELLULAR pH

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SUMMARY

(1) Changes of the holding potential applied to the membrane of myelinated nerve fibres induce slow variations of the peak sodium current, which are superimposed on the effect of sodium inactivation.

(2) These slow variations are transitions between various steady levels of available sodium conductance. Their time course can be described by the function erfc $(\sqrt{t/\tau})$ where t is the time and erfc the error function complement. The characteristic time τ lies in the range 2–4 min and depends on the membrane potential.

(3) Changes of extracellular pH cause a rapid change of the peak sodium current followed by a slow variation as observed after changes of the holding potential. This slow variation can be prevented by applying simultaneously an appropriate change of the holding potential, e.g. the effect of changing pH from 7.3 to 5.3 is balanced by changing the potential from −70 to −55 mV.

(4) The results are interpreted by postulating charged components diffusing slowly within the nodal membrane. Their transverse distribution controls the number of sodium channels available at a given membrane potential. The equivalence between change of pH and voltage is explained by assuming negative fixed charges at the outer surface of the membrane, which are protonated at low pH and thus affect the intrinsic membrane potential.

(5) It is concluded that effects which are ascribed to the action of agents on individual sodium channels have to be corrected for variations in the number of available channels if these agents influence the intrinsic membrane potential, e.g. changes of extracellular pH.

INTRODUCTION

In the preceding paper [1] an ultra-slow inactivation of the peak sodium current through the membrane of the node of Ranvier was described. It was shown that this current value approaches a new steady level when the holding potential or the ionic composition of the extracellular solution is changed. This transition

between various levels of available current is studied in this paper. Amplitude and time dependence of the transitions are analysed for various changes of holding potential and extracellular pH values. It has already been shown that the time course cannot be described by a single exponential function, but that a reasonable fit can be achieved by the sum of two exponentials [1]. The time constants of these exponentials, however, could not be related to the processes of normal (fast) [2] and slow [3] sodium inactivation in the node of Ranvier.

In this paper we show that the time dependence between various steady levels of available sodium conductance can be described by a single non-exponential function (error function of the square root of time). This function is derived from a model based on the electrodiffusion of charged components of the membrane, which are postulated to control the sodium conductivity.

METHODS

Motor and sensory fibres from the sciatic nerve of the frog *Rana esculenta* were investigated under voltage-clamp conditions as described in the preceding paper [1]. Membrane potentials V refer to the original holding potential, which was chosen at the beginning of the experiment to give a steady-state sodium inactivation of 30 %. Absolute membrane potentials E were calculated by assuming a value of -71 mV for this holding potential [4]. To record the peak sodium current, I_{Na}, the clamp potential was first set to $V = -40$ mV for 50 ms to remove sodium inactivation ($h_\infty = 1$). Then a test pulse of $V = +60$ mV was applied. The maximum inward current during this test pulse, corrected for leakage and baseline shifts, was taken as I_{Na}. These values were recorded in intervals of 10–30 s.

Ringer's solution was the same as used in the preceding paper [1]. Its pH was 7.3 at 15 °C. Solutions with reduced pH value were buffered with 5 mM sodium hydrogen phthalate and adjusted to the required pH values with HCl. The concentration of Na^+ was kept at 110 mM. Six test solutions were used with the following pH values (measured at 15 °C): 6.3, 5.8, 5.3, 4.8, 4.3 and 3.8. The extracellular solution superfusing the test node could be exchanged completely within 10 s.

Drifts of the holding potential were recorded as described in the preceding paper [1]. After lowering the extracellular pH from 7.3 to 5.3 a mean drift of +1.8 mV was observed, which is probably due to junction potentials.

RESULTS

(1) *Change of holding potential*

In Fig. 1 the peak sodium current I_{Na} is shown as a function of the time t_e after beginning of the experiment. The holding potential V_H was changed from 0 to -30 mV and 47 min later back to 0. After the step to the negative holding potential, the peak sodium current increases to a new steady level within 5 min (removal of ultra-slow sodium inactivation). This level is approximately stable for about 10 min, but decreases at longer times.

After switching back to $V_H = 0$ mV, the peak sodium current decreases first rapidly (reciprocal image of the onset), then more slowly and finally reaches the curve which is the continuation of the exponential decline corresponding to $V_H = 0$ (dashed curve in Fig. 1).

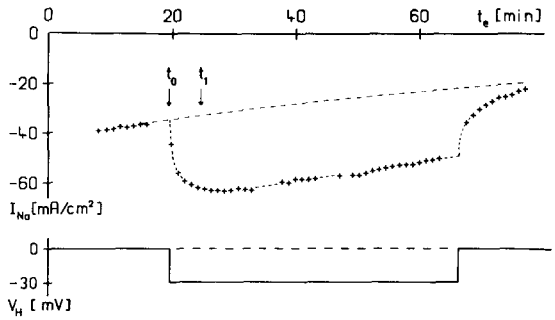

Fig. 1. Peak sodium current I_{Na} versus time t_e after beginning of the experiment. Changes of holding potential V_H are indicated in the lower part of the figure. Experiment F 55/73, motor fibre. Temperature 15 °C.

This investigation is concerned only with the transitions between the steady levels of the peak sodium current which occur within 5 min after a change of external parameters. The amplitude of the transition depends on the change of the holding potential and saturates at high hyperpolarizations. This voltage dependence was investigated in detail in the preceding paper (compare Fig. 4 of ref. 1). To study the time course of the transitions between the steady levels of the peak sodium current I_{Na}, we introduce normalized transitions $\Delta I(t)$ by

$$\Delta I(t) = \frac{I_{Na}(t) - I_{Na}^{\infty}}{I_{Na}(0) - I_{Na}^{\infty}} \tag{1}$$

where t is the time after changing the external parameter and I_{Na}^{∞} the steady level of I_{Na} under the new external conditions. This definition of $\Delta I(t)$ implies the normalization $\Delta I(0) = 1$ for positive and negative changes of the holding potential. If a measurable exponential decline occurs during the experiment, the differences $I_{Na}(t) - I_{Na}^{\infty}$ and $I_{Na}(0) - I_{Na}^{\infty}$ in Eqn. 1 have to be corrected for this decay. The exponential decline observed between 5 and 10 min after the change of V_H was extrapolated back to the point of change. I_{Na}^{∞} was assumed to follow this extrapolated exponential decline. As can be seen from Fig. 1, this correction is practically zero for steps to a more negative holding potential, but can become considerable in case of positive steps in holding potential. This fact causes high uncertainties in the determination of the characteristic time τ of decay of the peak sodium current after a step to a more positive holding potential or to a higher extracellular pH (see below), whereas this systematic error for steps to more negative holding potentials is mostly negligible.

Fig. 2 contains ΔI values obtained after changing the holding potential from $V_H = 0$ to $V_H = -30$ mV. They were calculated according to Eqn. 1 from the peak sodium current values I_{Na} of Fig. 1, found between $t_0 = 900$ s and $t_1 = 1226$ s and plotted versus $t = t_e - t_0$.

Two explore the time course of ΔI, the following three functions were tested which correspond to different relaxation mechanisms (see Discussion): $\exp(-t/\tau)$, $\operatorname{erfc}(\sqrt{t/\tau})$, $\exp(t/\tau) \cdot \operatorname{erfc}(\sqrt{t/\tau})$, where

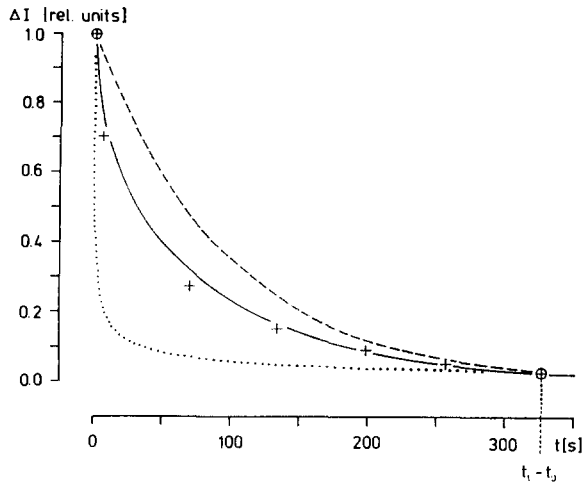

Fig. 2. (+) Normalized transitions ΔI between steady levels of the peak sodium current versus time t after switching the holding potential from 0 to −30 mV. Peak sodium currents I_{Na} are taken from Fig. 1 between $t_e = t_0$ and $t_e = t_1$. (- - -) exp $(-t/\tau)$; (—) erfc $(\sqrt{t/\tau})$; (. . .) exp $(t/\tau) \cdot$ erfc $(\sqrt{t/\tau})$. The characteristic time τ of each function is chosen to fit the ΔI value ⊕ at $t = t_1 - t_0$.

$$\text{erfc}(\xi) = 1 - \frac{2}{\sqrt{\pi}} \int_0^{\xi} \exp(-s^2) ds \tag{2}$$

is the error function complement. The characteristic time τ of each function was chosen to fit the ΔI value at $t - t_0$. It is obvious that $\Delta I(t)$ can only be described by the function erfc $(\sqrt{t/\tau})$. This non-exponential time course was found in all of 17 experiments with negative and positive changes of the holding potential.

Table I summarizes the characteristic time τ found for various changes of the holding potential V_H within the limits −30 mV and +20 mV. They were obtained by fitting the function erfc $(\sqrt{t/\tau})$ to normalized transitions between steady-state levels of the peak sodium current. τ is clearly voltage-dependent. For changes of the holding potential towards $V_H = 0$ and +20 mV, τ is approximately twice as large as for jumps towards the hyperpolarizing level $V_H = -30$ mV.

TABLE I

VALUES OF THE CHARACTERISTIC TIME τ OBTAINED BY FITTING THE TIME COURSE OF THE TRANSITIONS OF THE PEAK SODIUM CURRENT DUE TO CHANGES OF THE HOLDING POTENTIAL BY THE FUNCTION erfc $(\sqrt{t/\tau})$

Change of holding potential	τ (s)	Number of experiments
+20 mV → −30 mV	140	1
+15 mV → −30 mV	123±9	3
0 mV → −30 mV	115±9	5
−30 mV → 0 mV	202±36	5
0 mV → +20 mV	227±12	3

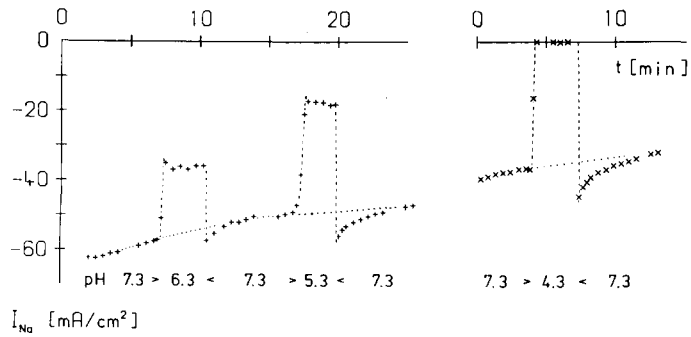

Fig. 3. Peak sodium current I_{Na} versus time t_e after beginning of the experiment for changes of extracellular pH as indicated in the lower part of the figure. (+) F 27/74 D; (×) F 28/74 D; both sensory fibres. Temperature 15 °C.

(2) *Change of extracellular pH*

Fig. 3 illustrates the time course of the peak sodium current after changes of the extracellular pH. After switching to a solution of low pH, the sodium current is immediately reduced owing to a partial block of sodium channels by protonation of specific charges at these channels (e.g. ref. 5). Thereafter the sodium current slowly increases to a steady level. A reverse effect occurs after the return to the normal pH of 7.3. The block of sodium channels is instantly removed and the sodium current is higher than the steady level before application of low pH. From there the peak sodium current declines within approximately 4 min to the steady level corresponding to a pH of 7.3.

The observed slow increase of the sodium current during low pH and its slow decay at high pH are similar to the transitions of the peak sodium current observed after switching to a more negative or a more positive holding potential, respectively (compare Fig. 1). Thus the action of hyperpolarization (depolarization) appears to be analogous to the effect of exposure to an extracellular solution of low (high) pH. The time courses of the after effects following a change of the holding potential or the extracellular pH are the same. In both cases the normalized transitions $\Delta I(t)$ are better described by the function erfc $(\sqrt{t/\tau})$ rather than by the relations exp $(-t/\tau)$ or exp $(t/\tau) \cdot$ erfc $(\sqrt{t/\tau})$. The characteristic time τ of the function erfc $(\sqrt{t/\tau})$ found to fit normalized transitions after changes of the extracellular pH varied between 30 and 100 s. Precise values cannot be obtained, because the rates of change of the sodium current after exchange of solution cannot be determined with sufficient accuracy. Therefore, no pH dependence of the characteristic time τ can be given.

The amplitude of the transition following an application of low pH depends on the pH chosen. In Fig. 3 it is larger after pH 4.3 than after pH 5.3 and 6.3. This feature was verified in all similar experiments.

(3) *Simultaneous change of pH and holding potential*

The preceding experiments suggest an equivalence between hyperpolarization (depolarization) and exposure to an extracellular solution of lowered (increased) pH. To confirm this equivalence quantitatively, experiments were performed in which

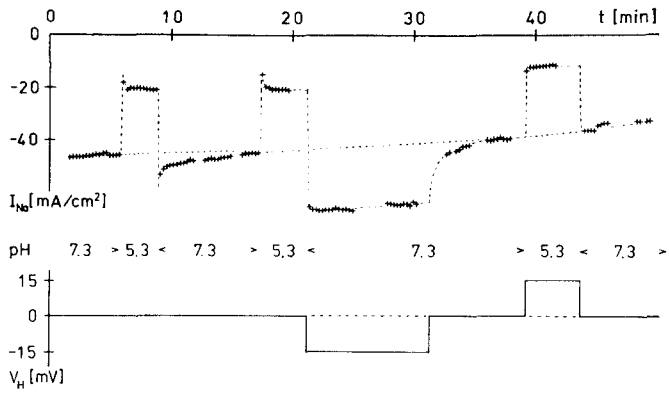

Fig. 4. Peak sodium current I_{Na} versus time t_e for simultaneous changes of the extracellular pH and holding potential V_H. Experiment F 22/74 D, sensory fibre. Temperature 15 °C.

the pH and the holding potential were simultaneously changed. Fig. 4 shows an example. A change of pH from 5.3 to 7.3 was combined with a hyperpolarization of −15 mV and the reverse pH change with a depolarization of + 15mV. The holding potential $V_H = -15$ mV switched on with the return to pH 7.3 prevents the decay of the enhanced level of the peak sodium current (excess current), which occurs at $V_H = 0$ (see Fig. 3 and first period of low pH of Fig. 4). Under these conditions no slow increase of the peak sodium current is found after the return to pH 7.3. The holding potential $V_H = +15$ mV applied during the action of pH 5.3 (third period of low pH in Fig. 4) prevents the slow increase of the size of the peak sodium current during that period (compare first and second periods at low pH of Fig. 4), and prevents the occurrence of an excess current after return to Ringer, which is normally found at $V_H = 0$. A depolarization of +15 mV, thus, compensates for the slow variations of the steady level of the peak sodium current due to the action of pH 5.3 and a hyperpolarization of −15 mV after the return to pH 7.3 sustains the steady level of the sodium current reached after unblocking the channels.

In separate experiments the change of pH from 7.3 to 5.3 was combined with holding potentials of $V_H = 20$ and 25 mV. In these cases the low steady level of the sodium current decreased instead of increasing ($V_H = 0$) or being unchanged ($V_H = 15$ mV). Corresponding negative holding potentials $V_H = -20$ and −30 mV switched on after the return to pH 7.3 led to a slow increase of the peak sodium current instead of no variation at $V_H = -15$ mV or a decay seen at $V_H = 0$ mV. The effect of pH 5.3, thus, was overcompensated by holding potentials exceeding $V_H = 15$ mV. A holding potential of approximately 15 mV, therefore, is equivalent to a pH change from 7.3 to 5.3. From similar experiments with various simultaneous changes of pH and holding potential, an equivalent voltage can be established for each pH difference. For a pH change from 7.3 to 4.3 we found the equivalent voltage to be approximately 30 mV.

The time and voltage dependence of the transitions of the peak sodium current did not show significant differences between motor and sensory fibres.

DISCUSSION

(1) *Equivalence between changes of extracellular pH and holding potential*

Excess sodium current in the nodal membrane after exposure to an extracellular solution of low pH or increased concentration of divalent cations was observed by several authors [1, 3, 5, 6]. This contribution also describes similar slow variations of the sodium current during exposure to low pH solutions (Fig. 3) and demonstrates that these transitions can be eliminated by switching the membrane potential to appropriate depolarizing or hyperpolarizing values (Fig. 4).

The small drift of the holding potential observed after lowering the extracellular pH excludes the possibility that the transitions arise from slowly decaying potentials at the electrodes or at the connecting agar bridges. Further, diffusion processes in the unstirred solution layers near the nodal membrane cannot account for the slow variations of the peak sodium current. In this case the change of two different external parameters (holding potential, extracellular pH) should give different time dependences of the sodium current, and the transitions induced by a shift of pH could not be compensated by a simultaneous change of the holding potential. The observed transitions between the steady levels of the peak sodium current, therefore, must be ascribed to a slowly changing sodium conductance of the nodal membrane.

The revealed equivalence between low pH and hyperpolarization or high pH and depolarization can be interpreted in terms of negative surface charges at the outside of the nodal membrane. Several recent publications [5, 7–9] confirm the existence of negative surface charges which are protonated at low pH values. Reducing the extracellular pH by the amount ΔpH causes a decrease $\Delta\Psi_0$ of the negative surface potential Ψ_0. The electric field strength in the membrane thus increases, though the external voltage is held constant at a value near the resting potential. This apparent hyperpolarization of the membrane can be compensated by a depolarizing change $\Delta V_H = -\Delta\Psi_0$ of the holding potential V_H. Thus the change $\Delta\Psi_0$ of the external surface potential Ψ_0 for a given pH difference ΔpH, can be determined by compensation experiments similar to that illustrated in Fig. 4.

The results ($\Delta\Psi_0 = 15$ mV for transitions from pH 7.3 to pH 5.3, $\Delta\Psi_0 = 30$ mV for transitions from pH 7.3 to pH 4.3) are in good agreement with the determination of $\Delta\Psi_0$ from shifts of sodium conductance voltage curves if these shifts are corrected for the voltage drop at the membrane series resistances (compare Fig. 5 of ref. 5). This equivalence between the compensation voltage and the shifts of the external surface potential indicates that the slow variations of the peak sodium current are controlled by a process depending on the electric field strength in the membrane.

(2) *Kinetics of the variation of peak sodium current*

We now turn to the investigation of the time dependence of transitions between steady levels of the peak sodium current after changing the holding potential or the extracellular pH. Similar slowly varying peak sodium currents were reported to occur in squid giant axons [10]. In these experiments the peak sodium current was measured as a function of the duration of long lasting hyperpolarizing prepulses. These currents increased with prepulse duration. A plateau of the peak sodium current was reached

with a time constant of about 100–500 ms and another plateau with a time constant in the range of 30–200 s [10]. In this work values between 30 and 250 s were found for the characteristic times of transitions between steady levels of the peak sodium current which, therefore, are comparable with the longer time constants measured in squid giant axons.

An essential feature of the transitions of the peak sodium current in the nodal membrane is that their time course cannot be described by a single exponential function (see Fig. 2). This non-exponential time dependence of transitions between steady levels of the peak sodium current excludes the possibility that these transitions arise from a transport of ions over a single energy barrier. Such a situation occurs if hydrophobic ions are incorporated into artificial lipid bilayer membranes. After a step change of the membrane potential, these ions would be redistributed between the energy minima at the membrane surfaces and the initial relaxation of the current would be found to be exponential [11].

On the other hand a time dependence proportional to $\exp(t/\tau) \cdot \operatorname{erfc}(\sqrt{t/\tau})$ would be the result of a relaxation of a diffusion process in the unstirred solution layers outside the membrane in the external solution or in the axoplasm [12, 13]. From Fig. 2 it is obvious, however, that neither function can describe the measured peak sodium currents. Instead, the relation $\operatorname{erfc}(\sqrt{t/\tau})$ seems to be appropriate. Such a time dependence is obtained mathematically as the result of an integrated diffusion equation including a term describing a recombination process with a relaxation time τ [14]. However, even this mechanism must be ruled out because τ then would be independent of the membrane voltage in contrast to the experimental results (compare Table I). The voltage dependence of τ thus requires a process occurring in the interior of the nodal membrane. In fact, the integration of the electrodiffusion equation under certain conditions leads to the function $\operatorname{erfc}(\sqrt{t/\tau})$ (see Appendix). Consequently, we postulate the existence of slowly diffusible, non-interacting charged components in the nodal membrane which control the sodium conductivity in the following way.

For negatively (positively) charged components we assume that an activation of the sodium current at depolarization is possible only if these components are located at the outer (inner) membrane surface. The peak sodium current during a depolarizing test pulse then will be proportional to the concentration of components at the appropriate membrane surface. If the holding potential is changed to a hyperpolarizing level, some components in the interior of the membrane are driven towards this surface and the sodium current increases. Correspondingly, a lower sodium current will be observed during continual depolarization. This mechanism, therefore, explains all properties of the "ultra slow sodium inactivation" which was described in the preceding paper [1]. In the proposed electrodiffusion model the time course of the surface concentration of components will be parallel to the magnitude of the peak sodium current. The calculation of this surface concentration is presented in the Appendix. For changes of the holding potential towards a hyperpolarization of $V_H = -30$ mV, the normalized transitions ΔI between the steady levels of the peak sodium current can be described by

$$\Delta I(t) = \operatorname{erfc}\left(\frac{e}{2kTd}|zU|\sqrt{Dt}\right) \tag{3}$$

where D and z are diffusion coefficient and valence of the charged components in the membrane, e is the elementary charge, k is Boltzmann constant and T is the absolute temperature. U is the voltage across the membrane phase of thickness d and is related to the externally applied voltage E by

$$U = E - \Psi_o + \Psi_i \tag{4}$$

Of the surface potentials Ψ_o and Ψ_i at the outer and inner surface of the nodal membrane, only Ψ_o can be determined directly from shifts of conductance voltage curves in experiments with various ionic compositions of the external solution. In this way $\Psi_o = -48$ mV was obtained for pH = 7.3 of the external solution [5]. For the inner surface potential Ψ_i, at the membrane of squid giant axons a lower value of -17 mV was determined [15]. Thus, taking $\Psi_o = -48$ mV and $\Psi_i = -17$ mV, we obtain $U = E + 31$ mV. At the resting potential $E = E_r = -71$ mV [4] it is $U = -40$ mV. Zero electric field in the interior of the membrane ($U = 0$) would be obtained at $E = -31$ mV or at a depolarization of $V = E - E_r = +40$ mV.

For changes of the holding potential from $V_H = -30$ to 0 mV and from 0 to $+20$ mV the time course of normalized transitions of the peak sodium current also can be described by the function erfc $(\sqrt{t/\tau})$ (see Table I and Appendix, Eqn. 18).

To give an estimate of the diffusion coefficient of the charged components we use the values of the characteristic time τ compiled in Table I. Since the scatter of the data for steps of the holding potential to -30 mV is lower than for depolarizing steps we consider only the former case for which Eqn. 3 is appropriate. Equating with erfc $(\sqrt{t/\tau})$ yields the characteristic time

$$\tau = \frac{1}{D}\left(\frac{2kTd}{zeU}\right)^2 \tag{5}$$

for the transitions as a function of the membrane parameters d, z, D and U. This relation predicts that τ should depend only on the voltage U after the change of the holding potential and not on the preceding voltage. This is confirmed by the results of the first three rows of Table I. An average value of $\tau = 120 \pm 6$ s is obtained from these nine experiments in which the holding potential was switched to $V_H = -30$ mV ($U = -72$ mV). Assuming a membrane thickness of $d = 50$ Å, the diffusion coefficient D becomes $D \approx 10^{-15}/z^2 \cdot \text{cm}^2 \cdot \text{s}^{-1}$ at 15 °C. The valence z of the charged components cannot be determined from our experiments. Only a lower limit can be deduced from the value $z' = 2.1 \pm 0.4$ of the effective valence (z times the fraction of the membrane potential acting on the components) which was derived from the voltage dependence of the ultra-slow sodium inactivation [1]. Since the absolute value of the actual charge must be higher than the effective valence, it is $|z| \geqslant 2$. According to the model, the diffusion coefficient D describes the transverse migration of the charged components through the nodal membrane. Its very low value indicates a macromolecular size of the postulated membrane components which control the sodium conductance.

Diffusion coefficients of the order of 10^{-16} cm$^2 \cdot$ s^{-1} are not unrealistic. Values of this magnitude were reported for the diffusion in metals and in non-polar crystals (ref. 21, page 260). Further, measurements of the rate of inside-outside transitions (flip-flop) of spin-labeled phospholipids provide evidence for a slow

transverse diffusion through membranes. In membrane vesicles prepared from the electroplax of *Electrophorus electricus* a half time of 3.8–7 min of flip-flop was measured [16] corresponding to a traverse diffusion coefficient in the range of 10^{-15} $cm^2 \cdot s^{-1}$.

CONCLUSION

To describe the magnitude and time course of the transitions between the steady levels of the peak sodium current before and after a change of the holding potential or the extracellular pH, it was postulated that the sodium conductance depends on the location of charged components in the interior of the nodal membrane. The spatial distribution of these components controls the maximum sodium conductance [1], which is proportional to the number of functioning sodium channels. If this interpretation is correct, the peak sodium currents under different experimental situations should be compared only at identical numbers of functioning sodium channels.

This conclusion affects the results previously derived for the binding constant of protons at the specific charges of the sodium channels [5] and the number of ultraviolet-sensitive sodium channels at low pH values [17]. After lowering the extracellular pH, the number of functioning sodium channels slowly increases, parallel to the magnitude of the peak sodium current, and reaches a stationary value after approx. 1.5 min (see Figs. 3 and 4). Identical numbers of functioning sodium channels, therefore, are present if the last current value at high pH and the initial value at low pH are taken. After changes from pH 7.3 to pH 5.3, the initial peak sodium current at pH 5.3 is approx. 20 % lower than the steady level after 2 min if the holding potential is held constant at $V_H = 0$ (compare Figs. 3 and 4). Therefore, more sodium channels are blocked at low pH than the number obtained by comparing steady sodium current levels. Taking this correction into account, the pK_H value for the binding of protons at the specific charges of the sodium channels will be approximately 4.7, 0.2 units higher than that derived recently [5].

According to the same correction the ultraviolet sensitivity of H^+-blocked sodium channels is higher than that determined from steady current values [17]. In particular, the sensitivity values at low pH relative to the sensitivity measured at pH 7.3 (open channels) have to be increased. Quantitative data will be presented in a separate communication.

Similar corrections must be applied to the results of all previous investigations in which blocking effects in ion selective channels were derived from a comparison of steady current levels in experiments with solutions of different ionic compositions. The most precise way of correction would be to eliminate the transitions of the peak sodium current by a suitable change of the holding potential (see Fig. 4).

Furthermore, all sodium conductances measured at different holding potentials have to be corrected for the effects of ultra-slow sodium inactivation.

APPENDIX: DERIVATION OF Eqn. 3

The aim is to calculate the time dependence of the surface concentration of charged membrane components after a step change of the membrane potential.

Flux ϕ and concentration c of these components in the interior of the membrane are connected by the one-dimensional Nernst-Planck equation

$$\phi = -D\left(\frac{\partial c}{\partial x} + \frac{zeU}{kTd}c\right) \tag{6}$$

where x is the coordinate normal to the membrane, and $x = 0$ is the surface under consideration. D and z are diffusion coefficient and valence of the membrane components. Since the profile of the electrical potential in the nodal membrane is unknown, for simplicity we assume a constant electric field strength U/d in the membrane where U is the voltage across the membrane phase and d is its thickness. This constant field approximation is valid if the concentration of univalent ions in the membrane is less than $5 \cdot 10^{-5}$ M [18].

By combining the Nernst-Planck equation (Eqn. 6) with the equation of continuity

$$\frac{\partial \phi}{\partial x} = -\frac{\partial c}{\partial t} \tag{7}$$

the flux ϕ can be eliminated:

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial x^2} + \frac{zeU}{kTd}\cdot\frac{\partial c}{\partial x}\right) \tag{8}$$

Further, it is postulated that the diffusing components are confined to the membrane phase. This gives the boundary condition $\phi = 0$ at $x = 0$:

$$\frac{\partial c}{\partial x} + \frac{zeU}{kTd}\cdot c = 0;\ (x = 0) \tag{9}$$

A similar relation would have to be formulated for the opposite membrane surface $x = d$. However, it can be expected that for times t smaller than the diffusion time τ_D across the membrane, processes at one interface are irrelevant to those occurring at the other. Calculating the concentration changes at the interface $x = 0$ we, therefore, can neglect the boundary condition at $x = d$ and treat the membrane as an infinite phase, if $t < \tau_D$. As an estimate for τ_D we use the expression $\tau_D = d^2/(\pi^2 D)$ which is valid for zero electric field in the membrane. It was shown by Cole [19, 20] that τ_D is a good approximation to the relaxation time of redistribution processes at arbitrary membrane potentials. With $D = 10^{-15}/z^2 \cdot \mathrm{cm}^2 \cdot \mathrm{s}^{-1}$ (see Discussion) and with a membrane thickness of $d = 50$ Å, $\tau_D = 25 \cdot z^2$ s is obtained. The omission of the boundary condition at $x = d$ thus implies that the following expressions are valid for times $t < 25 \cdot z^2$ s after a step of the membrane potential ($|z| \geqslant 2$, see above). If at time $t = 0$ the voltage across the membrane phase is stepped from U_o to U, it is $\phi = 0$ for $t \leqslant 0$ and the initial concentration profile at $t = 0$ becomes:

$$c(x, 0) = c_o \exp\left(-\frac{zeU_o}{kTd}\cdot x\right);\ (x \geqslant 0) \tag{10}$$

The integration constant c_o is the initial surface concentration. To solve the partial differential equation (Eqn. 8) together with the boundary conditions (Eqns. 9 and 10) we introduce the transformation (ref. 21, page 47)

$$c(x, t) = c^*(x, t) \exp\left[-\frac{zeU}{2kTd}x - \left(\frac{zeU}{2kTd}\right)^2 Dt\right] \tag{11}$$

and obtain for $c^*(x, t)$ the ordinary diffusion equation

$$\frac{\partial c^*}{\partial t} = D\frac{\partial^2 c^*}{\partial x^2} \tag{12}$$

instead of Eqn. 8.

The solution of this equation together with the appropriate boundary conditions for $c^*(x, t)$ is straightforward by employing the method of Laplace transformation (for an illustration of this method compare ref. 22).

The final result for the time dependence of the concentration $c(0, t)$ at the surface $x = 0$ reads:

$$c(0, t) = \frac{c_o}{2}\frac{U}{U_o}\left[T_1(t) - T_2(t)\right] \tag{13}$$

with

$$T_1(t) = \operatorname{erfc}\left(-\frac{zeU}{2kTd}\sqrt{Dt}\right) \tag{14}$$

$$T_2(t) = \left(1 - \frac{2U_o}{U}\right)\exp\left[\left(\frac{ze}{kTd}\right)^2 U_o(U_o - U)Dt\right] \cdot \operatorname{erfc}\left[\frac{ze}{kTd}\left(U_o - \frac{U}{2}\right)\sqrt{Dt}\right] \tag{15}$$

The voltage U across the membrane phase is related to the externally applied voltage E by: $U = E + 31$ mV (compare Discussion).

For an estimate of the magnitude of the terms $T_1(t)$ and $T_2(t)$ we calculate the expression

$$\Delta = \frac{T_2(0) - T_2(\tau_D)}{T_1(0) - T_1(\tau_D)} \tag{16}$$

for a change of the holding potential $V_H = E_H - E_r = E_H + 71$ mV from $V_H = 0$ ($U_o = -40$ mV) to $V_H = -30$ mV ($U = -70$ mV) and for various values of the valence z. Δ is smaller than 16 % for any value of z. Thus the time course of $c(0, t)$ between $t = 0$ and $t = \tau_D$ is mainly determined by the first term $T_1(t)$ on the right-hand side of Eqn. 13. Similarly, the term $T_2(t)$ can be neglected for changes of the holding potential from +15 or +20 mV to −30 mV. Therefore, the normalized transitions $\Delta I(t)$ of the peak sodium current, which are postulated to be proportional to the surface concentration $c(0, t)$, are given by Eqn. 3 of the Discussion:

$$\Delta I(t) = \operatorname{erfc}\left(\frac{e}{2kTd}|zU|\sqrt{Dt}\right) \tag{17}$$

For changes of the holding potential from −30 to 0 mV and from 0 to +20 mV the second term $T_2(t)$ on the right-hand side of Eqn. 13 gives the dominant contribution to the time dependence of $c(0, t)$. In these cases the exponential factor $\exp[(ze/kTd)^2 U_o \cdot (U_o - U)Dt]$ varies more slowly than $\operatorname{erfc}[ze(U_o - U/2)\sqrt{Dt}/kTd]$ between $t = 0$ and $T = \tau_D$. $\Delta I(t)$ is then approximately

$$\Delta I(t) = \operatorname{erfc}\left(\frac{e}{2kTd}|z(U - 2U_o)|\sqrt{Dt}\right) \tag{18}$$

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