INCREASED CHARGE DISPLACEMENT IN THE MEMBRANE OF MYELINATED NERVE AT REDUCED EXTRACELLULAR pH

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ABSTRACT Asymmetry currents were measured in nodes of myelinated nerve fibers from *Rana esculenta* at extracellular pH values of 5.2, 7.0, and 8.1 by averaging the currents during and after 1-ms depolarizing and hyperpolarizing voltage pulses. The charge displacement in the nodal membrane was obtained by numerical integration of the asymmetry currents. Lowering the pH from 7.0 to 5.2 significantly slows down the kinetics of the fast charge displacement during depolarization but hardly affects the kinetics after repolarization. The pH reduction increases the maximum charge displacement during depolarization by 46%. No differences between asymmetry currents were found between pH 7.0 and 8.1. It is concluded that protonation by extracellular H^+ ions may increase the net charge or the transition range of mobile subunits in the nerve membrane.

INTRODUCTION

In recent years an asymmetrical charge displacement has been detected in nerve membranes and attributed to the movement of gates in the sodium channels (see reviews by Almers, 1978; Armstrong and Bezanilla, 1975; Meves, 1977; Neumcke et al., 1978). The relation between charge displacement and gating is supported by the simultaneous blockage of asymmetry and sodium currents by various chemical agents (e.g., Zn, glutaraldehyde, D_2O, procaine), by scorpion venom (Nonner, 1979), and by ultraviolet radiation (Fox et al., 1976).

Sodium channels in nerve can also be blocked by extracellular protons (Hille, 1968; Drouin and Neumcke, 1974; Carbone et al., 1978) which are assumed to bind either to the outer channel mouth (Neumcke, 1977) or in the interior of the channel (Woodhull, 1973), but not directly to the gating subunits. The finding that the asymmetrical charge displacement in squid giant axons is unaffected by the extracellular pH over the range 5–8 (Keynes and Rojas, 1974) would be in agreement with these assumptions. However, in myelinated nerve significant modifications of the charge displacement in the nodal membrane occur at reduced extracellular pH. The present paper describes measurements of asymmetry currents in myelinated nerve fibers at different pH values. We find that lowering the pH from 7.0 to 5.2 significantly increases the magnitude and time constant of the fast charge displacement during depolarization.

METHODS

Single myelinated nerve fibers of *Rana esculenta* were studied under voltage clamp conditions at 15°C. In previous publications the dissection of single nerve fibers (Stämpfli and Hille, 1976), the method of

voltage clamping (Nonner, 1969), and the measurement and analysis of asymmetry currents (Neumcke et al., 1976) were described in detail.

The fibers were cut in an artificial axoplasm solution containing 120 mM CsCl. For measuring asymmetry currents the node was superfused with extracellular test solutions composed of 105 mM tetramethylammonium (TMA) chloride, 10 mM tetraethylammonium chloride, 10 mM buffer, 1.8 mM $CaCl_2$, and 300 nM tetrodotoxin. Test solutions of pH 7.0 and 8.1 were buffered with morpholinopropanesulfonic acid (MOPS), the test solution of pH 5.2 with morpholinoethanesulfonic acid (MES), and all solutions were titrated with TMA·OH to the desired pH value. The junction potentials of the test solutions of pH 5.2, 7.0, and 8.1 with respect to isotonic CsCl were 0.1, 0.5, and 1.1 mV, respectively (test solutions positive).

Displacements of the membrane potential from its resting value (where 30% of the sodium channels were inactivated in Ringer's solution) are always denoted by V. At pH 7.0 the fibers were held at the holding potential $V_H = -20$ mV. Lowering the extracellular pH to 5.2 reduces the amount of the negative outer surface potential at the nodal membrane by ~16 mV (see Fig. 5 of Drouin and Neumcke, 1974). Therefore, the fibers were held at $V_H = -4$ mV at pH 5.2 to compensate for the corresponding change of the electric field strength in the membrane. At pH 8.1 a holding potential of −24 mV was chosen.

Membrane currents were calibrated using a longitudinal axoplasm resistance determined from electrical measurements (Chiu et al., 1979). The currents were measured on-line every 10 μs during a 1-ms test pulse, applied from the holding potential, and within 0.5 ms after repolarization to the holding potential. To compensate the leakage and linear capacity current an appropriate analogue signal was subtracted. The remaining current samples during and after a positive test pulse V and during and after two negative test pulses $V_H - (V - V_H)/2$ of the same duration but of one-half the size of the positive pulse were added to obtain asymmetry currents. The interval between pulses was 0.5 s, and the samples from 64 positive and 128 negative pulses were averaged. With this procedure asymmetry currents were measured for depolarizations between $V = 20$ and 120 mV in steps of 20 mV.

The measured asymmetry current was integrated numerically during the test pulse ("on" response) and after repolarization to the holding potential ("off" response) to obtain the asymmetrical charge displacement $Q(t)$. In both cases the last 10–20 $Q(t)$ values were linearly increasing or decreasing with time t and thus could be described as $Q(t) = Q + a \cdot t$. The term $a \cdot t$ represents possible asymmetries of ionic currents and slow components of charge displacements. The early $Q(t)$ values of "on" and "off" responses were then fitted to the expression

$$Q(t) = Q[1 - \exp(-t/\tau)] + a \cdot t. \quad (1)$$

Though a single exponential function did not always adequately describe the early $Q(t)$ values, the fitted time constants τ, nevertheless, were used to characterize the kinetics of the fast charge displacement during and after the test pulses.

Due to immobilization of mobile charges during depolarizing test pulses, the fast charge displacement, Q_{off}, of the "off" response, is smaller than the corresponding quantity, Q_{on}, of the "on" response (Nonner et al., 1978). The degree of immobilization varies with the length and height of the pulse and could be pH dependent as well. Thus, comparison of Q_{off} values at different pH does not seem meaningful; we therefore concentrate in the following on the pH dependence of Q_{on} only.

RESULTS

Lowering the extracellular pH from 7.0 to 5.2 slows the kinetics of asymmetry currents during depolarizing test pulses. Fig. 1 illustrates this behavior for depolarizations of 60 and 100 mV. On the other hand, the kinetics of the "off" response is hardly pH dependent (compare τ values listed in the legend to Fig. 1). In the experiment illustrated in Fig. 1 the sequence of applied extracellular pH values was 7.0, 5.2. In other experiments the pH sequence was

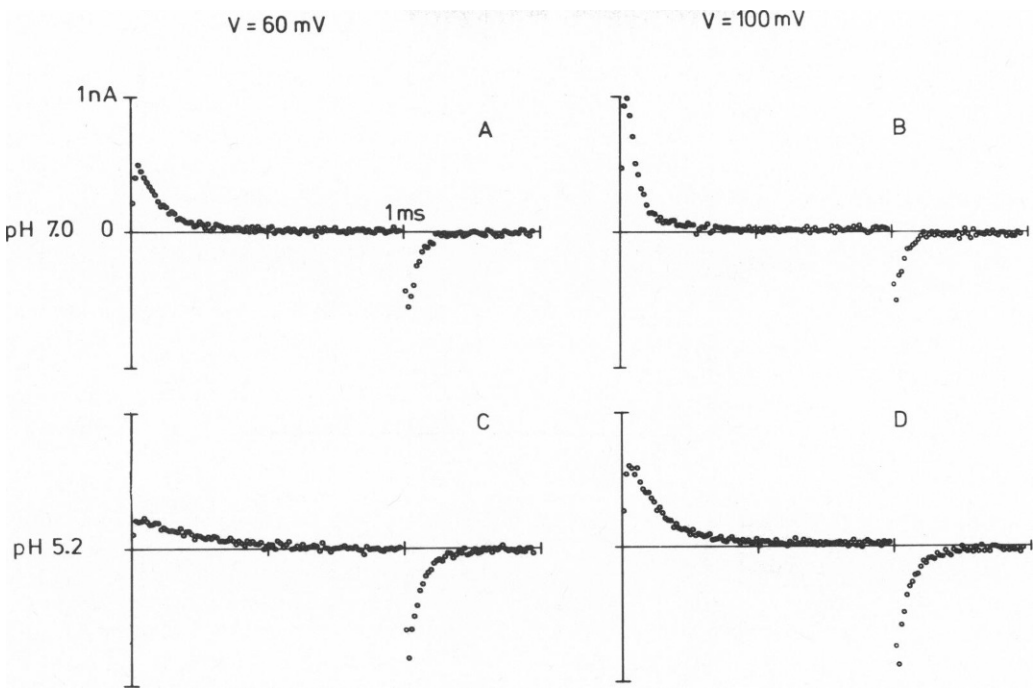

FIGURE 1 Asymmetry currents during and after 1-ms depolarizations to V = 60 and 100 mV at pH 7.0 (*A*, *B*) and 5.2 (*C*, *D*). Units of abscissa and ordinate as indicated in *A*. The corresponding fast charge displacements Q_{on} and Q_{off} in *fC* (= 10^{-15} As) and the time constants τ_{on} and τ_{off} in μs are as follows:

	Q_{on}	τ_{on}	Q_{off}	τ_{off}
(*A*) pH 7.0, V = 60 mV:	55.1	117	−29.9	47.1
(*B*) pH 7.0, V = 100 mV:	68.9	68.6	−22.3	41.3
(*C*) pH 5.2, V = 60 mV:	58.8	271	−46.3	56.1
(*D*) pH 5.2, V = 100 mV:	84.7	139	−47.9	54.6

Note increase of Q_{on} at reduced pH despite reduced peak of asymmetry current. Experiment G 17/79, motor fiber. Temperature, 15°C.

reversed without affecting the result. Also, no systematic differences were found between motor and sensory fibers.

Mean values of time constants τ_{on} and τ_{off} at depolarizations between 20 and 120 mV are plotted in Fig. 2. It is obvious that at depolarizations where $V \geq 60$ mV the τ_{on} values at pH 5.2 are about twice as large as those at pH 7.0, whereas only a small increase is observed for τ_{off}. Fig. 2 *A* also reveals a shift of the maximum of τ_{on} towards higher depolarizations at reduced pH. A shift of about the same amount is also seen in the voltage dependence of the fast charge displacement during the "on" response (arrows in Fig. 3). Since the absolute charges Q_{on} scatter between different experiments, they were normalized to the value of V = 120 mV at pH 7.0.

It may be surprising that the Q_{on} values at pH 5.2 and $V > 60$ mV exceed the corresponding charge values at pH 7.0, as the asymmetry currents in Fig. 1 are always smaller at reduced pH. However, the kinetics of the asymmetry currents at pH 5.2 is so much slower that the

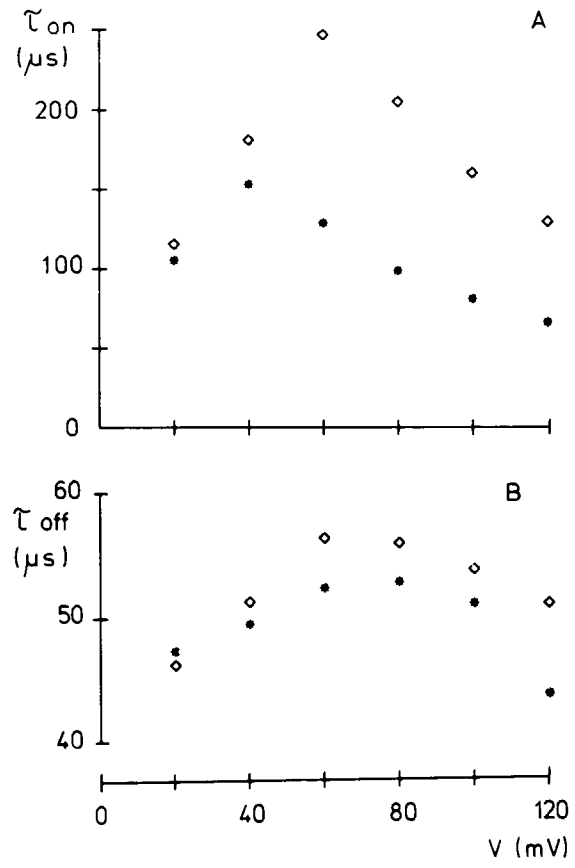

FIGURE 2 Time constants of fast charge displacement as function of depolarization V at pH 7.0 (*) and 5.2 (◇). (*A*) Time constant τ_{on} of "on" response. (*B*) Time constant τ_{off} of "off" response. Symbols represent mean values from seven experiments. Temperature, 15°C.

area under the "on" response becomes larger with respect to that at pH 7.0 (compare also Q_{on} values listed in the legend to Fig. 1).

At pH 5.2 the fast charge displacement, Q_{off}, after test pulses, is also larger and the immobilization of mobile charges during depolarizations proceeds slower than at pH 7 (Fig. 1). These pH effects on the "off" response were not analyzed in detail.

The curves in Fig. 3 were calculated from the function

$$Q(V) = \frac{Q_{max}}{1 + \exp\left[\frac{-z'e(V - V_0)}{kT}\right]}, \tag{2}$$

which was fitted to the Q_{on} values (e, elementary charge; k, Boltzmann constant; T, absolute temperature: kT/e = 24.8 mV at 15°C). The function may be interpreted as the voltage dependence of the distribution of mobile charges between two discrete states (Keynes and Rojas, 1974). The function is characterized by three parameters: the maximum charge

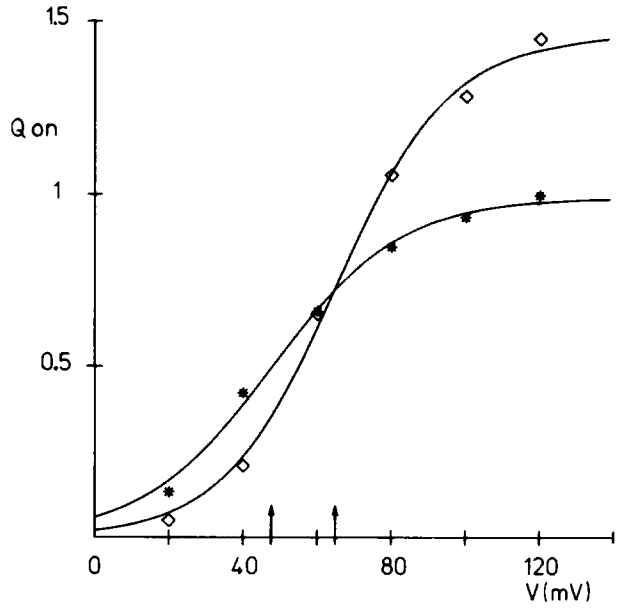

FIGURE 3 Magnitude Q_{on} of fast charge displacement of "on" response as function of depolarization V at pH 7.0 (*) and 5.2 (◇). Q_{on} values were obtained by fitting the asymmetrical charge displacement to Eq. 1 and normalizing them to the value at V = 120 mV, pH 7.0. Symbols represent mean values from seven experiments. The curves represent the charge distribution function (Eq. 2) fitted to the Q_{on} values. The locations of the midpoints of the curves are indicated by arrows on the abscissa.

displacement, Q_{max}; the midpoint potential, V_0; and the effective valence, z', of charges—that is, their actual charge multiplied by the fraction of the voltage drop across the membrane phase acting between the two states. Table I contains the values of the three parameters at pH 7.0 and 5.2.

If instead of a charge distribution between two states, multiple transitions of charges between many states are assumed (Meves, 1974, Neumcke et al., 1978), a three times higher effective valence z' is derived by fitting the appropriate charge distribution function to the Q_{on} values.

In two separate experiments asymmetry currents were measured during and after depolarizations between 20 and 120 mV at extracellular pH values of 7.0 and 8.1. No systematic differences between both pH values were found.

TABLE I
PARAMETERS OF CHARGE DISTRIBUTION FUNCTION (EQ. 2), (MEAN ± SEM, SEVEN EXPERIMENTS)

	pH 7.0	pH 5.2
Q_{max}	0.995 ± 0.031	1.457 ± 0.048
V_0 (*millivolts*)	47.6 ± 2.1	64.7 ± 1.9
z'	1.43 ± 0.16	1.64 ± 0.16

DISCUSSION

Our experiments reveal a pronounced dependence of the asymmetry currents in myelinated nerve on the extracellular pH, contrary to the results of experiments by Keynes and Rojas (1974) performed on squid giant axons. This discrepancy could result from a difference in species or from experimental difficulties in detecting pH effects on squid giant axons. In this preparation the action of an extracellular pH change on membrane currents develops slowly, and a steady state is only reached after several minutes.[1] In myelinated nerve fibers, however, the pH effects are complete immediately after a change of solution.

There could be two possible reasons for the increase of the fast charge displacement at reduced extracellular pH: either the net charge of the mobile membrane subunits could be enhanced, or the subunits could make transitions over a broader mobility range within the membrane and thus could experience a larger fraction of the voltage drop across the membrane phase. In both cases the observed increase of the maximum charge displacement, Q_{max}, and of the effective valence, z', of charges could be explained. Thus, our results do not prove that extracellular protons bind directly to the mobile membrane subunits, even though this would be the easiest way to explain the pH dependence of the charge displacement. In this case the mobile subunits at pH 7 would have to bear a net positive charge, as otherwise the charge displacement would decrease in lowered pH.

Reducing the extracellular pH from 7.0 to 5.2 increases the maximum charge displacement, Q_{max}, by 46%, but the effective valence, z', of mobile charges only by 15%. The discrepancy could be due to the use of Eq. 2, which does not adequately describe the charge displacement, or it could originate from the recruitment of mobile charges at reduced pH.

The shift of the voltage dependence of the time constant τ_{on} (Fig. 2 *A*) and of the charge values Q_{on} (Fig. 3) to higher depolarizations at reduced extracellular pH may be attributed to a corresponding change of the outer surface potential: the shift of the midpoint potentials V_0 of the curves in Fig. 3 by 17 mV is in perfect agreement with a change of the surface potential by 16 mV between pH 7.0 and 5.2 deduced from previous experiments (Drouin and Neumcke, 1974). Differences in liquid junction potentials between test solutions of pH 7.0 and 5.2 are below 1 mV (see Methods) and thus can be neglected. In our experiments the difference in surface potential was compensated by a corresponding change of the holding potential ($V_H = -20$ mV at pH 7, $V_H = -4$ mV at pH 5.2) to have the same electric field strength in the membrane under holding conditions. A possible small nonlinear charge displacement produced by the negative test pulses would then be of similar magnitude at both pH values, and thus would not affect the increase of the net charge displacement at reduced extracellular pH, as shown in Fig. 3.

No significant alterations of asymmetry currents could be detected when the extracellular pH was changed from 7.0 to 8.1. This indicates that the degree of ionization of the charges affecting the displacement of mobile membrane subunits is constant within this pH range.

Lowering the extracellular pH significantly slows the kinetics of the fast charge displacement during depolarization (Fig. 2 *A*), but hardly affects the kinetics after repolarization to the holding potential (Fig. 2 *B*). The change of τ_{on} is comparable with an increase of the time constants of sodium activation and inactivation observed in squid giant axons (Carbone et al.,

[1]Carbone, E. Personal communication.

1978) and in myelinated nerve fibers (Drouin and Neumcke, unpublished data). This might suggest a fluidity decrease of the membrane phase at reduced extracellular pH (Watts et al., 1978) which, however, does not affect the time constant τ_{off} to the same extent.

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REFERENCES

ALMERS, W. 1978. Gating currents and charge movements in excitable membranes. *Rev. Physiol. Biochem. Pharmacol.* **82**:96–190.

ARMSTRONG, C. M., and F. BEZANILLA. 1975. Currents associated with the ionic gating structures in nerve membrane. *Ann. N. Y. Acad. Sci.* **264**:265–277.

CARBONE, E., R. FIORAVANTI, G. F. PRESTIPINO, and E. WANKE. 1978. The action of extracellular pH on Na^+ and K^+ membrane currents in the giant axon of "Loligo vulgaris." *J. Membr. Biol.* **43**:295–315.

CHIU, S. Y., J. M. RITCHIE, R. B. ROGART, and D. STAGG. 1979. A quantitative description of membrane currents in rabbit myelinated nerve. *J. Physiol. (Lond.).* **292**:149–166.

DROUIN, H., and B. NEUMCKE. 1974. Specific and unspecific charges at the sodium channels of the nerve membrane. *Pfluegers Arch. Eur. J. Physiol.* **351**:207–229.

FOX J. M., B. NEUMCKE, W. NONNER, and R. STÄMPFLI. 1976. Block of gating currents by ultraviolet radiation in the membrane of myelinated nerve. *Pfluegers Arch. Eur. J. Physiol.* **364**:143–145.

HILLE, B. 1968. Charges and potentials at the nerve surface. Divalent ions and pH. *J. Gen. Physiol.* **51**:221–236.

KEYNES, R. D., and E. ROJAS. 1974. Kinetics and steady-state properties of the charged system controlling sodium conductance in the squid giant axon. *J. Physiol. (Lond.).* **239**:393–434.

MEVES, H. 1974. The effect of holding potential on the asymmetry currents in squid giant axons. *J. Physiol. (Lond.).* **243**:847–867.

MEVES, H. 1977. Activation, inactivation, and chemical blockage of the gating current in squid giant axon. *Ann. N. Y. Acad. Sci.* **303**:332–338.

NEUMCKE, B. 1977. Specific and non-specific surface charges at nerve membranes. *In* Electrical Phenomena at the Biological Membrane Level. E. Roux, editor. Elsevier Scientific Publishing Company, Amsterdam-Oxford-New York. 257–272.

NEUMCKE, B., W. NONNER, and R. STÄMPFLI. 1976. Asymmetrical displacement current and its relation with the activation of sodium current in the membrane of frog myelinated nerve. *Pfluegers Arch. Eur. J. Physiol.* **363**:193–203.

NEUMCKE, B., W. NONNER, and R. STÄMPFLI. 1978. Gating currents in excitable membranes. *In* International Review of Biochemistry. Vol. 19. J. C. Metcalfe, editor. University Park Press, Baltimore. 129–155.

NONNER, W. 1969. A new voltage clamp method for Ranvier nodes. *Pfluegers Arch. Eur. J. Physiol.* **309**:176–192.

NONNER, W. 1979. Effects of Leiurus scorpion venom on the "gating" current in myelinated nerve. *Adv. Cytopharmacol.* **3**:345–352.

NONNER, W., E. ROJAS, and R. STÄMPFLI. 1978. Asymmetrical displacement currents in the membrane of frog myelinated nerve: early time course and effects of membrane potential. *Pfluegers Arch. Eur. J. Physiol.* **375**:75–85.

STÄMPFLI, R., and B. HILLE. 1976. Electrophysiology of the peripheral myelinated nerve. *In* Frog Neurobiology. R. Llinás and W. Precht, editors. Springer-Verlag, Berlin-Heidelberg. 3–32.

WATTS, A., K. HARLOS, W. MASCHKE, and D. MARSH. 1978. Control of the structure and fluidity of phosphatidylglycerol bilayers by pH titration. *Biochim. Biophys. Acta* **510**:63–74.

WOODHULL, A. M. 1973. Ionic blockage of sodium channels in nerve. *J. Gen. Physiol.* **61**:687–708.